Molecular systematics and taxonomy of *Sporacestra* and relatives (Ramalinaceae, Ascomycota)

Malin Stapnes Dahl

Master of Science thesis

Natural History Museum
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Abstract

Tropical members of the Ramalinaceae remain poorly studied. In this master thesis, I have addressed the phylogenetic relationship within a clade of genera occurring in the Tropics and Subtropics, including members of *Bacidia, Bacidiopsora, Phyllopsora* and a resurrected *Sporacestra.* A phylogeny based on a nuclear (ITS) and a mitochondrial (SSU) DNA-region is presented. Bayesian and parsimony phylogenetic analyses on a total of 57 taxa, supports the monophyly of *Sporacestra*. *Bacidia* s. lat. was recovered as polyphyletic. Members of *Bacidiopsora* and *Phyllopsora* were nested into a monophyletic *Bacidia* s. str. Based on the molecular phylogenetic results, I describe three new species in *Sporacestra* and lump *Bacidiopsora* and one member of *Phyllopsora* into *Bacidia* s. str. According to the phylogenetic hypothesis presented, thallus architecture and secondary chemistry proved be a poor diagnostic character at the genus level, being present in multiple genera in the Ramalinaceae. Five new species, *Bacidia corallina* Stapnes & Timdal, *B. kariegae* Stapnes, *Sporacestra isidata* Stapnes & Timdal, *S. longispora* Stapnes and *S. straminea* Stapnes are described. *Phyllopsora borbonica* is reduced to synonymy with *P. pertexta,* and a new combination of this species, *S. pertexta* (Nyl.) Stapnes & Timdal is presented. Two new names are given, namely *Bacidia microphylla* Stapnes and *B. soralifera* Stapnes & Timdal. A key to *Sporacestra* is provided.

Acknowledgements

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For the fieldwork in Venezuela, I would like to thank Jesus Hernandez and his students at Universidad Central de Venezuela. I am grateful for the exchange of knowledge and all your hospitality. Without your help, fieldwork in Venezuela would not be possible to carry out.

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1 Introduction

The majority of all undescribed fungal species, including lichenized fungi, are expected in habitats such as tropical forests (Hawksworth & Rossman, 1997; Lawrey & Diederich, 2003; Sipman & Aptroot, 2001). Of the ca. 2 million geocoded collections and observations of the Lecanoromycetes (the largest class of lichenized fungi, containing 78% of all accepted lichenized species [Lücking et al., 2016]) in GBIF, only 3% are from the tropics, and only 0.08% from the Amazon (E. Timdal, pers. comm.). This clearly shows how under-explored the tropical habitat is, in particular the Amazon, and that it is likely that many species are still left to be discovered.

Tropical members of the lichen family Ramalinaceae remain poorly studied. A set of genera occurring almost exclusively in the Tropics and Subtropics are currently being studied using molecular methods by Timdal and co-workers. An unpublished preliminary phylogenetic study based on the mitochondrial small subunit (mtSSU) revealed several strongly supported clades within the Ramalinaceae (Fig. 1: clade A; Bendiksby & Timdal, unpubl.). One of those, here referred to as clade A, consists of a few species currently included in the three genera: (1) the tropical genus *Phyllopsora* Müll. Arg. (the type species *P. breviuscula*

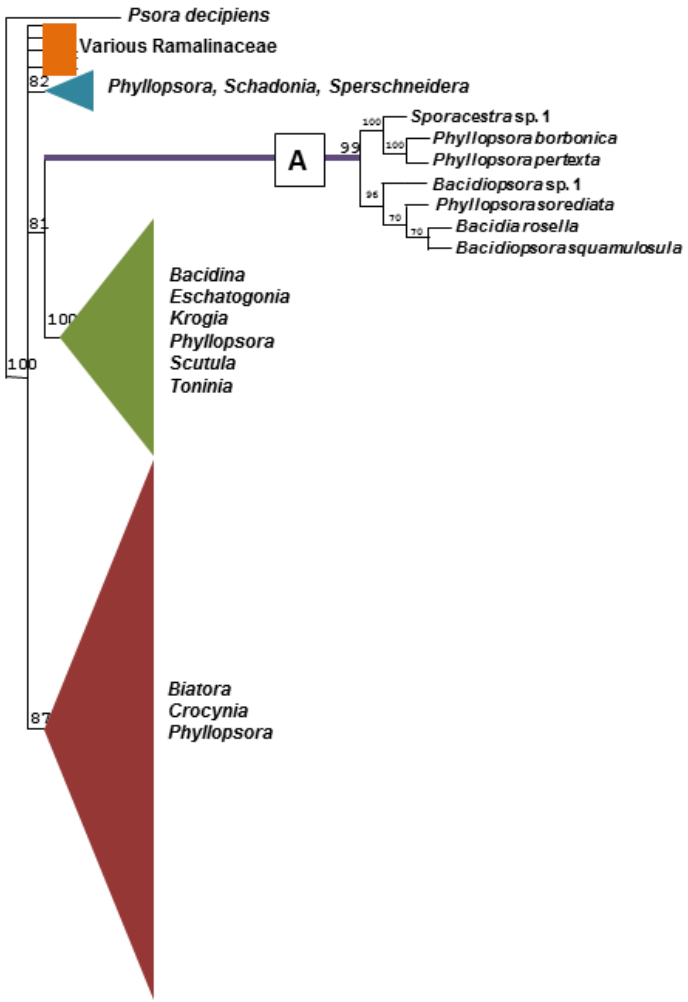

Fig. 1: Parsimony jackknife concensus phylogeny of the Ramalinaceae with preliminary data (mtSSU only) by Bendiksby & Timdal (unpublished). Clade A, consisting of species currently included in *Bacidia, Bacidiopsora, Phyllopsora* and tentatively determined to *Sporacestra,* is the basis of this thesis.

(Nyl.) Müll. Arg excluded), (2) the temperate and tropical genus *Bacidia* De Not. (the type *B. rosella* included; for author citations of the species see Table 1), and (3) the tropical genus *Bacidiopsora* Kalb (the type *B. squamulosula* included). One indetermined species assumed by Bendiksby & Timdal (unpubl.) to be included in a resurrected genus *Sporacestra* A. Massal was also included in clade A. This long unused genus name is typified on *Biatora prasina* Tuck. & Mont. (nom. illeg.), a species later placed in *Bacidia* by Coppins (1983). In Ekman (1996: p. 43) it is stated that: "*In fact, the only species included in* Sporacestra *by Massalongo,* Biatora prasina *Tuck. & Mont. (a nomen illegitimum), is synonymous with* Psorella pertexta, *a fact which has been overlooked up until now.* [….] *I have seen the types of nearly all South and Central American* Bacidia *s. lat. with long and septate spores, but no species is close to* Psorella pertexta*"* , where *Psorella pertexta* (Nyl.) Müll. Arg. is a synonym of *Phyllopsora pertexta* (Nyl.) Swinscow & Krog. On the basis of this, it was assumed by Bendiksby & Timdal (unpubl.) that *Sporacestra* should be resurrected. Clade A (Fig. 1) is the topic of this master thesis.

Bacidia is a large genus of crustose species with a world-wide distribution. It consists of c. 230 species (Lücking et al., 2016), most of them corticolous. The main diagnostic character for the genus is the morphology of the ascospores: thick-walled and multiseptate. The most comprehensive study of the genus to date is the PhD thesis on all species of *Bacidia* and *Bacidina* in North America by Ekman (1996). A search in the *Recent Literature on Lichens* database (http://nhm2.uio.no/lichens) displays 386 publications on *Bacidia*, most of which are taxonomic papers or checklists. In a molecular study based on the nuclear ribosomal internal transcribed spacer (ITS), monophyly of *Bacidia* was rejected, with members of *Bacidina* Vézda and *Toninia* A. Massal. nested into *Bacidia* (Ekman, 2001). There is no study on *Bacidia* to date where *Bacidiopsora* or *Phyllopsora* are included. At the start of this study, there were 157 DNA sequence entries of 29 *Bacidia* species in GenBank.

Bacidiopsora is a tropical/subtropical genus consisting of six rarely collected species (Lücking et al., 2016). When sterile, *Bacidiopsora* species are more or less morphologically indistinguishable from *Phyllopsora* in the field. When fertile, the apothecia are more flattened and with a better developed margin. The main distinguishing characters however, are in the ascospores: thick-walled and multi-septate in *Bacidiopsora*, and thin-walled and simple in *Phyllopsora*. Moreover, *Bacidiopsora* differs from *Bacidia* in forming a squamulose thallus and containing hyperhomosekikaic and homosekikaic acid, whereas *Bacidia* mostly forms a

crustose thallus and contains no lichen substances or rarely atranorin and zeorin (Ekman, 1996; Kalb & Elix, 1995). There are few studies on *Bacidiopsora* (A. Aptroot, 2002; Brako, 1991; Kalb, 1988, 2004; Kalb & Elix, 1995), most of which are reports on new species, including discussions about its relationship to other members of the Ramalinaceae based on morphology, anatomy and chemistry. No molecular studies are published to this date and therefore no DNA sequence entries of *Bacidiopsora* exist in GenBank.

Phyllopsora is a genus consisting of crustose to squamulose species, occurring primarily in Tropical and Subtropical humid woodland and rainforests. More than 120 species names exist in the literature (Timdal, 2011). The genus has been revised by Swinscow and Krog (1981), Brako (1989, 1991), Timdal and Krog (2001), and Timdal (2008, 2011), but only with morphological, anatomical and chemical methods. A preliminary molecular phylogenetic analysis of a broad sampling of *Phyllopsora* species and selected presumed relatives (Bendiksby & Timdal, unpubl.: Fig. 1) indicates that the genus is highly polyphyletic. A molecular phylogenetic study on the genus as a whole is in preparation by Kistenich et al.

Phyllopsora borbonica and *P. pertexta* belong in clade A (Fig. 1). The former is Paleotropical, the latter Neotropical. It has been indicated by anatomical studies (Timdal, 2011) that the two species may in-fact be synonymous. Comparing the species' descriptions, the latter differs from the former mainly in the pigmentation in the hypothecium within the ascocarp (cfr. Swinscow & Krog, 1981; Timdal & Krog, 2001).

Brako (1989) indicated that *P. pertexta* belonged in an undescribed genus together with *P. cognata* (Nyl.) Timdal and *P. microphyllina* (Nyl.) Swinscow & Krog, due to anatomical differences separating them from *Phyllopsora.* Timdal (2008, 2011) applied a broader genus concept for *Phyllopsora*, regarding the anatomical differences outlined by Brako (1989) not being sufficient for splitting the genus. Unfortunately, *P. cognata* and *P. microphyllina* are both unambiguously known only from their type collections (Cuba, localities unknown, leg. C. Wright, in the 1850s or early 1860s).

A third *Phyllopsora* species, *P. sorediata,* also seems to be related to *Bacidia* and *Bacidiopsora* (Fig. 1: clade A). The species was originally described in the genus *Triclinum* Fée by Aptroot et al. (2007), which Timdal (2011) regarded as synonymous with the genus *Phyllopsora.* The type species of *Triclinum*, *P. cinchonarum* (Fée) Timdal, currently studied by Kistenich and coworkers, seems to belong in a different clade within the Ramalinaceae

(S. Kistenich, pers. comm.). Hence, the generic position of *P. sorediata* is in need of revision, as it is neither a *Phyllopsora* nor a *Triclinum.*

The aim with the present study is to reveal phylogenetic relationships within clade A (Fig. 1) and to use this phylogenetic hypothesis to assess the taxonomic affiliation and species delimitations in this clade. With basis in the preliminary molecular phylogeny by Bendiksby & Timdal (unpubl.), I will expand the dataset, both with regard to taxon sampling and genetic data, in order to increase resolution and support for phylogenetic relationships. Attaining an integrative taxonomic approach (Dayrat, 2005), I will investigate the morphology, anatomy, and chemistry of clade A. Moreover, I will relate these data to the molecular phylogenetic hypothesis in the work towards potential taxonomic changes. Some obvious hypotheses to be tested include: (1) *Sporacestra* represents a monophyletic genus; (2) *Phyllopsora borbonica* is synonymous with *P. pertexta*; (3) *Bacidiopsora* sp. 1 represents a new genus; (4) *Phyllopsora sorediata* represents a new genus; (5) *Bacidiopsora* is monophyletic; (6) *Bacidiopsora* is not nested within *Bacidia*.

Table 1: *Specimens used in this study with voucher information (extraction numbe r, species names, countr y, location, collector, collection number or herbaria number with herbaria location, type information, major lichen substances and GeneBank accession number or symbol indicating a newly generated sequence of either mtSSU or ITS). Bold face indicates that the sequence or chemistry data a re generated in this study.*

EXTR. #	SPECIES	COUNTRY	LOCATION	COLLECTOR	COLL.# or Hb.#(HB)	TYPE	CHEMISTRY	mtSSU	ITS
-	*Bacidia absistens* (Nyl.) Arnold	Norway		Ekman, S.	3223 (BG)			SE93	SE93
-	*Bacidia arceutina* (Ach.) Rehm & Arnold	Sweden		Ekman, S.	3110 (BG)			SE23	SE23
-	*Bacidia auerswaldii* (Hepp ex Stinzenb.) Mig.	Sweden		Johansson	20 (UPS)				AF282122
-	*Bacidia bagliettoana* A. Massal & De not.	Sweden		Ekman, S.	3137 (BG)				AF282123
-	*Bacidia beckhausii* Körber	India		Prithiviraj, B. & Hariharan, G. N.	"MSSRF Lichen Herbarium"				JF714252
-	*Bacidia biatorina* (Körber) Vaini	Sweden		Knutsson	94-148 (hb Knutsson)				AF282079
-	*Bacidia caligans* (Nyl.) A. L. Sm **1**	Sweden		Johansson	21 (UPS)				AF282096
-	*Bacidia caligans* **2**	Austria		-	-			EU516945	
-	*Bacidia diffracta* Ekman	USA		Wetmore	26401 (MIN)				AF282090
-	*Bacidia fraxinea* Ekman & Nordin	Sweden		Johansson	1620 (BG)				AF282088
-	*Bacidia hemipolia* (Th. Fr.) Malme	USA		Tønnsberg	25091 (BG)				AF282072
-	*Bacidia illudens* (Nyl.) Lange	-		Westberg & Devon	LMK033 (LD)			SE306	SE306
-	*Bacidia incompta* (Hooker) Anzi	Sweden		Ekman, S.	3144(BG)			SE117	SE117
-	*Bacidia laurocerasi* ssp. *laurocerasi* (Del. Ex Duby) Vaini	USA		Wetmore	78318 (MIN)				AF282078
-	*Bacidia lutescens* Malme	USA		Ekman, S.	L-1193 (LD)				AF282082
-	*Bacidia polychroa* (Th. Fr.) Körber	Sweden		Knutsson	91-215 (hb Knutsson)				AF282089
-	*Bacidia rosella* (Pers.) De not.	-		Ekman, S.	3117 (BG)	Type species of *Bacidia*		SE50	SE50
-	*Bacidia rubella (*Hoofm.) A. Massal.	-		-	-			DQ986808	HQ650644
-	*Bacidia schweinitzii* (Tuck.) A. Schneid.	USA	North Carolina	Lendemer, Lutzoni	30548 (NY), 0047624 (DUKE)			DQ972998	KX151761
-	*Bacidia scopulina* (Nyl.) A. L. Sm.	Sweden		Ekman, S.	3106 (BG)				AF282084
-	*Bacidia sipmanii* M. Brand, Coppins, Van den Boom & Sérus	Tenerife		Sérusiaux	361 (LG)			JQ796832	
4802	*Bacidia* sp.	Brazil		Dahl, Kistenich, Timdal & Toreskaas	L-202582 (O)		**NONE**	-	**X2***
4803	*Bacidia* sp.	Brazil	Pará, Thailândia, Fazenda Agroecológica São Roque	Dahl, Kistenich, Timdal & Toreskaas	L-202612 (O)		**NONE**	**X***	**X2***
4807	*Bacidia* sp.	Venezuela	Pará, Thailândia, Fazenda Agroecológica São Roque	Dahl, Kistenich, Timdal & Toreskaas	L-202555 (O)			**XB*, X3R*, XR***	**X***
4808	*Bacidia* sp.	Venezuela	Carabobo, Parque Nacional Henry Pittier	Dahl, Kistenich, Timdal & Toreskaas	L-202543 (O)		**ARG**	-	**X2***
4809	*Bacidia* sp.	Venezuela	Capital District, Parque Nacional Macarao	Dahl, Kistenich, Timdal & Toreskaas	L-202524 (O)		**GYR**	**XB***	-
4810	*Bacidia* sp.	Venezuela	Capital District, Parque Nacional Macarao	Dahl, Kistenich, Timdal & Toreskaas	L-202461 (O)		**GYR**	**X***	**X***
4811	*Bacidia* sp.	Brazil	Miranda, Caracas, El Volcan	Dahl, Kistenich, Timdal & Toreskaas	L-202693 (O)		**NONE**	**XR***	-
4812	*Bacidia* sp.	Brazil	Rio de Janeiro, Parque Nacional do Itatiaia	Dahl, Kistenich, Timdal & Toreskaas	L-202686 (O)		**NONE**	**XR***	**X***
5627	*Bacidia* sp.	Brazil	Rio de Janeiro, Parque Nacional do Itatiaia	Dahl, Kistenich, Timdal & Toreskaas	L-202818 (O)		**UNKN**	**X***	**X2***
5629	*Bacidia* sp.	Brazil	Rio de Janeiro, Parque Nacional do Itatiaia	Dahl, Kistenich, Timdal & Toreskaas	L-202777 (O)		**NONE**	**XF***	-
5630	*Bacidia* sp.	Brazil	São Paulo, Parque Estadual das Fontes do Ipiranga	Dahl, Kistenich, Timdal & Toreskaas	L-202842 (O)		**NONE**	**X***	-
5633	*Bacidia* sp.	Brazil	Rio de Janeiro, Parque Nacional do Itatiaia	Dahl, Kistenich, Timdal & Toreskaas	L-202792 (O)		**ARG**	**XA***	-
6045	*Bacidia* sp.	Venezuela	Rio de Janeiro, Parque Nacional do Itatiaia	Dahl, Kistenich, Timdal & Toreskaas	L-202456 (O)		**NONE**	**X***	-
6047	*Bacidia* sp.	Brazil	Miranda, Caracas, El Volcan	Dahl, Kistenich, Timdal & Toreskaas	L-202602 (O)		**NONE**	**X***	-
6072	*Bacidia* sp.	Brazil	Pará, Thailândia, Fazenda Agroecológica São Roque	Dahl, Kistenich, Timdal & Toreskaas	L-202679 (O)		**UNKN**	**XB***	-
-	*Bacidia* sp. **1**	Switzerland	Pará, Thailândia, Fazenda Agroecológica São Roque	-	-				KX098339

Abbrevations: Extr.# = extraction number, Coll.# = collection number, Hb.# = herbarium number.
*X = sequence successfully generated, letters or numbers following X indicates that only parts of the DNA were successfully sequenced; 1 = only ITS1, 2 = only ITS2, 4 = only ITS 4, 5 = only ITS 5, 3R = only mtSSU3R, A = only mtSSUA, B = only mtSSUB , F = only mtSSU-F, LR = only ITS-lichR, R = only mtssuR, * indicates that the sequence is not included in this study, - indicates that there were no successfull amplificiation of DNA.*

Table 1: *continued.*

EXTR. #	SPECIES	COUNTRY	LOCATION	COLLECTOR	COLL.# or Hb.#(HB)	TYPE	CHEMISTRY	mtSSU	ITS
-	*Bacidia* sp. **2**	Switerland		-	-				KX098340
-	*Bacidia subincompta* (Nyl. Arnold) **1**	Switerland		-	-				KX098342
-	*Bacidia subincompta* **2**	Sweden		Ekman, S.	3413 (BG)				AF282125
-	*Bacidia suffusa* (Fr.) Schneider	-		Lumbsch, H., -	1919c, -			KJ766355	AF282091
-	*Bacidia vermifera* (Nyl.) Th. Fr. **1**	-		-	-				AF282109
-	*Bacidia vermifera* **2**	United Kingdom		CJ Ellis & BJ Coppins	L629: 13 (-)				FR799129
5761	*Bacidia wellingtonii* (Stirt.) D. J. Galloway	New Zealand	Waian River, Manapouri	Ziviagina, N.	L-204598 (O)	Type species of *Psorella*		X	
6030	*Bacidiopsora microphyllina* Kalb	Costa Rica	Puntarenas	Kalb, K. & Plöbst, G.	122188 (WIS)		HSEK	-	-
6034	*Bacidiopsora microphyllina*	Venezuela	Tachira	Kalb, K. & Kalb, A.	122197 (WIS)		HHSEK	-	-
6035	*Bacidiopsora microphyllina*	Cameroon	North West province, Mount Oku	Frisch, A. & Tamnjong Idi	122195 (WIS)		HHSEK, HSEK	-	-
6036	*Bacidiopsora microphyllina*	Réunion	-	Kalb, K. & Kalb, A.	122196 (WIS)		HSEK	-	-
6033	*Bacidiopsora microphyllina*	Brazil	Minas Gerais	Kalb, K. & Plöbst, G.	122186 (WIS)		HHSEK, HSEK	-	-
6073	*Bacidiopsora microphyllina*	South Africa	Eastern Cape, Western District, 11 km NNW of Kenton-on-Sea	Rui, S. & Timdal, E.	L-202868 (O)		**HHSEK/HSEK**	**XB**	**X1**
6074	*Bacidiopsora microphyllina*	South Africa	Eastern Cape, Western District, 11 km NNW of Kenton-on-Sea	Rui, S. & Timdal, E.	L-202867 (O)		**HHSEK/HSEK**	**XB**	**X1**
6011	*Bacidiopsora orizabana* (Vain.) Kalb	Thailand	Doi Chiang Dao	Allen, D.	764974 (BM)		**NONE**	**XB***	-
6013	*Bacidiopsora psorina* (Nyl.) Kalb	Sri Lanka	-	Aptroot, A.	733846 (BM)			-	-
6026	*Bacidiopsora psorina*	Venezuela	Merida	Kalb, K., Kalb, A. & López-Figueiras	122194 (WIS)		HHSEK, HSEK, SEK	-	-
6027	*Bacidiopsora psorina*	Ecuador	Azuay	Kalb, K. & Kalb, A.	122192 (WIS)		HHSEK, HSEK, SEK	**XB***	**X2***
6028	*Bacidiopsora psorina*	Guatemala	Chimaltenango	Kalb, K. & Plöbst, G.	122193 (WIS)		HHSEK, HSEK	-	**X2***
6029	*Bacidiopsora psorina*	Brazil	Minas Gerais	Kalb, K. & Plöbst, G.	122190 (WIS)		HHSEK, HSEK	-	-
6031	*Bacidiopsora silvicola* (malme) Kalb	Brazil	São Paulo	Kalb, K. & Plöbst, G.	122187 (WIS)		HSEK	-	-
6032	*Bacidiopsora silvicola*	Ecuador	Azuay	Kalb, K. & Kalb, A.	122189 (WIS)		HHSEK, HSEK	-	-
6075	*Bacidiopsora* sp.	Venezuela	Miranda, Caracas, El Volcan	Dahl, Kistenich, Timdal & Toreskaas	L-202459 (O)		**UNKN**	**X***	**X***
6076	*Bacidiopsora* sp.	Venezuela	Miranda, Caracas, El Volcan	Dahl, Kistenich, Timdal & Toreskaas	L-202458 (O)		**NONE**	**X***	-
6080	*Bacidiopsora* sp.	South Africa	Mpumalanga, Ehlanzeni District, Buffelkloof Nature Reserve	Burrows, J. & Timdal, E.	L-196765 (O)		**HHSEK/HSEK**	**X**	-
4805	*Bacidiopsora* sp.	South Africa	Mpumalanga, Ehlanzeni District, grassland above Sudwala Caves	Burrows, J. & Timdal, E.	L-196740 (O)		**HHSEK**	-	-
4806	*Bacidiopsora* sp.	South Africa	Mpumalanga, Ehlanzeni District, Buffelkloof Nature Reserve	Burrows, J. & Timdal, E.	L-196810 (O)		**HHSEK/HSEK**	**XB***	-
1014	*Bacidiopsora* sp. 1	Thailand	Uthai Thani, Khlong Plou	Aguirre, James & Wolseley	2478a (BM)		**HHSEK/HSEK, XANTH**	X	X
1015	*Bacidiopsora* sp. 1	Thailand	Uthai Thani, Khao Nang Rum	Aguirre, James & Wolseley	749853 (BM)		**HHSEK/HSEK, XANTH**	X	
1428	*Bacidiopsora* sp. 1	Thailand	Uthai Thani, Khlong Plou	Aguirre, James & Wolseley	1031544 (BM)		**HHSEK/HSEK, XANTH**	X	
6037	*Bacidiopsora* sp. 2	South Africa	Mpumalanga, Ehlanzeni District, Buffelkloof Nature Reserve	Burrows, J. & Timdal, E.	L-196747 (O)		**HHSEK/HSEK**	**X**	-
6078	*Bacidiopsora* sp. 2	South Africa	Mpumalanga, Ehlanzeni District, Buffelkloof Nature Reserve	Burrows, J. & Timdal, E.	L-196757 (O)		**HHSEK/HSEK**	**XB**	-
6079	*Bacidiopsora* sp. 2	South Africa	Mpumalanga, Ehlanzeni District, Buffelkloof Nature Reserve	Burrows, J. & Timdal, E.	L-196762 (O)		**HHSEK/HSEK**	**XB**	-
6040	*Bacidiopsora* sp. 3	Venezuela	Capital District, Parque Nacional Waraira Repano	Dahl, Kistenich, Timdal & Toreskaas	L-202485 (O)		**HHSEK/HSEK**	**X**	**X**
6041	*Bacidiopsora* sp. 3	Venezuela	Capital District, Parque Nacional Waraira Repano	Dahl, Kistenich, Timdal & Toreskaas	L-202484 (O)		**HHSEK/HSEK**	**X**	**X**
6023	*Bacidiopsora squamulosula* (Nyl.) Kalb.	Costa Rica	San José	Kalb, K. & Plöbst, G.	122200 (WIS)	Type species of *Bacidiopsora*	HHSEK, HSEK, SEK	-	-
6025	*Bacidiopsora squamulosula*	Ecuador	Azuay	Kalb, K. & Kalb, A.	122201 (WIS)		HHSEK, HSEK, SEK	**XB***	-
6024	*Bacidiopsora squamulosula*	Brazil	Minas Gerais	Kalb, K. & Plöbst, G.	122199 (WIS)		HHSEK, HSEK, SEK	-	-
508	*Bacidiopsora squamulosula*	Ecuador	Azuay	Kalb, K. & Kalb, A.	L-113543 (O)		**HHSEK/HSEK**	X	X

Table 1: *continued.*

EXTR. #	SPECIES	COUNTRY	LOCATION	COLLECTOR	COLL.# or Hb.#(HB)	TYPE	CHEMISTRY	mtSSU	ITS
5636	*Bacidiopsora tenuisecta* (Vain.) Aptroot	Brazil	Catas Altas, Parque Natural do Caraca	Aptroot, A.	5030069994830 (BR)		**HHSEK/HSEK**	-	-
6017	*Bacidiospora orizabana*	Thailand	Uthai Thani, Khao Nang Rum	Aguirre, James & Wolseley	763699 (BM)			-	-
5483	*Luekingia polyspora* Aptroot & Umana	Costa Rica	Limón, Hitoy Cerere Reserve, near Pandrora,	Aptroot, A.	5030070745698 (BR)	IT of *L. polyspora*		X	
511	*Phyllopsora borbonica* Timdal & Krog	La Réunion	along road towards Plaine d'Affouches, above Bras Citron	Krog, H. & Timdal, E.	L-797 (O)	HT of *P. borbonica*	**NONE**	X	X
6012	*Phyllopsora borbonica*	Seychelles	Mahé, Mare aux cochons	Beaver, K.	114307 (hb Seaward)		**NONE**	-	-
427	*Phyllopsora borbonica*	Indonesia	Kalimantan Selan, Hulu Tabalong, site 4	Wolseley	T13LQ (BM)		**NONE**	XA	X2
6021	*Phyllopsora confusa* Swinscow & Krog	Cameroon	North West province, Donga-Mantunga Division	Frisch, A. & Tamnjong Idi	99/Ka4210 (hb A. Frisch)		**TERP**	**XB***	-
1040	*Phyllopsora pertexta* (Nyl.) Swinscow & Krog	Cuba	Pinar del Río,La Palma, Mogote el Pan de Guajaibón	Pérez-Ortega, S.	s.n. (hb Pérez-Ortega)		**NONE**	X	**X**
5638	*Phyllopsora pertexta*	Papua New Guinea	Morobe, Herzog Mts, Wago	Jeremy, A.C.	577345 (BM)		**NONE**	-	-
5641	*Phyllopsora pertexta*	Papua New Guinea	Morobe, Bululo	Hill, D. J.	921669 (BM)		**NONE**	**XB***	-
6015	*Phyllopsora pertexta*	Papua New Guinea	Morobe, Bewapi	Jeremy, A.C.	577330 (BM)		**NONE**	-	-
6016	*Phyllopsora pertexta*	Papua New Guinea	Morobe, Mt. Selk	Hill, D. J.	921683 (BM)		**NONE**	-	-
6043	*Phyllopsora pertexta*	Venezuela	Capital District, Parque Nacional Waraira Repano	Dahl, Kistenich, Timdal & Toreskaas	L-202494 (O)		**FUR**	**XB***	-
6044	*Phyllopsora pertexta*	Venezuela	Capital District, Parque Nacional Macarao	Dahl, Kistenich, Timdal & Toreskaas	L-202519 (O)		**ATR**	**XB***	**X***
6085	*Phyllopsora pertexta*	Brazil	Rio Abajo	Cáceres, M.	20131 (ISE)		**NONE**	**XB***	-
6087	*Phyllopsora pertexta*	Brazil	Rio Abajo	Cáceres, M.	20134 (ISE)		**NONE**	**XB**	**X**
6086	*Phyllopsora pertexta*	Brazil	Rio Abajo	Cáceres, M.	20110 (ISE)		**NONE**	**XB***	-
5622	*Phyllopsora soralifera* Timdal	Brazil	Pará, Paragominas, Hydro mining area	Barbosa, R.S., Haugan, R. & Timdal, E.	L-193972 (O)		**NONE**	-	-
5623	*Phyllopsora soralifera*	Brazil	Pará, Paragominas, Hydro mining area	Barbosa, R.S., Haugan, R. & Timdal, E.	L-193924 (O)		**NONE**	**X***	**X2***
5624	*Phyllopsora soralifera*	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã,	Kistenich, S. & Timdal, E.	L-201089 (O)		**NONE**	**X***	**X1*, X4***
1433	*Phyllopsora sorediata* (Aptroot & Sparrius) Timdal	South Africa	Eastern Cape, Grootrivier	Nordin, A.	L-092604 (UPS)		**DIV, ARG**	X	
5637	*Phyllopsora sorediata*	Thailand	Uthai Thani, Khlong Plou	Aguirre, James & Wolseley	763640 (BM)		**DIV, ARG**	**XB***	-
1008	*Phyllopsora sorediata*	Thailand	Uthai Thani, Khlong Plou	Aguirre, James & Wolseley	749391 (BM)		**DIV, ARG**	X	
1007	*Phyllopsora sorediata*	Thailand	Uthai Thani, Khao Nang Rum	Aguirre & Wolseley	749765 (BM)		**DIV, ARG**	X	
6009	*Phyllopsora* sp.	Malaysia	Borneo, Sabah, Maliau Basin	Thüs, Wolseley &Vairappan	M087 (BORH)		**NONE**	-	-
6010	*Phyllopsora* sp.	Malaysia	Borneo, Sabah, SAFE-project Area	Thüs, Wolseley &Vairappan	S.E.02.2 (BORH)		**NONE**	**XB***	-
6014	*Phyllopsora* sp.	Brazil	Rio Abajo	Cáceres, M.	20109 (ISE)		**TERP**	**XB***	-
6019	*Phyllopsora* sp.	Cameroon	East province, Yokaduma	Frisch, A. & Tamnjong Idi	99/Ka2774 (hb A. Frisch)		**NONE**	-	-
6020	*Phyllopsora* sp.	Tanzania	Morogoro Region	Frisch, A.	99/Tz354 (hb A. Frisch)		**NONE**	**XB***	-
6022	*Phyllopsora* sp.	Brazil	Rio Abajo	Cáceres, M.	20107 (ISE)		**TERP**	**X***	**X5***
1312	*Physcidia cylindrophora*	Taiwan	Taichung co. Mt. Yuanazuei	Ohmura, Y.	7091 (GZU)			X*	X*
395	*Physcidia* sp.	Cuba	Holguín, Moa, Parque Nacional Alejandro de Humboldt	Buck, W.R.	1149477 (NY)		**ATR, ZEO**	X	
6084	*Physcidia* sp.	Malaysia	Borneo, Sabah, Maliau Basin	Vairappan, C.	L504 (BM)			**XB***	-
5613	Ramalinaceae indet.	Brazil	Pará, Paragominas, Hydro mining area	Barbosa, R.S., Haugan, R. & Timdal, E.	L-193872 (O)		**TERP**	**XB***	**X2***
5615	Ramalinaceae indet.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201039 (O)		**NONE**	**X3***	**X***
5616	Ramalinaceae indet.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201091 (O)		**NONE**	-	-
5620	Ramalinaceae indet.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201092 (O)		**ARG**	**XB***	**X2***
5625	Ramalinaceae indet.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201021 (O)		**NONE**	**XA***	**X2***
5639	Ramalinaceae indet.	Cameroon	South west provice, Fako Division	Frisch, A. & Tamnjong Idi	99/Ka1204 (hb A. Frisch)		**UNKN**	-	-
5640	Ramalinaceae indet.	Cameroon	Littoral Province, Douala-Edea Forest Reserve	Frisch, A. & Tamnjong Idi	99/Ka2528 (hb A. Frisch)		**NONE**	-	-
6018	Ramalinaceae indet.	Cameroon	North West province, Donga-Mantunga Division	Frisch, A. & Tamnjong Idi	99/Ka4209 (hb A. Frisch)		**NONE**	-	-

Abbrivations: IT = Isotype, HT = Holotype

Table 1: *continued.*

EXTR. #	SPECIES	COUNTRY	LOCATION	COLLECTOR	COLL.# or Hb.#(HB)	TYPE	CHEMISTRY	mtSSU	ITS
6042	Ramalinaceae	Venezuela	Miranda, Caracas, El Volcan	Dahl, Kistenich, Timdal & Toreskaas	L-202457 (O)		**NONE**	**X***	**X1***
6077	Ramalinaceae	Malaysia	Borneo, Sabah, Maliau Basin	Vairappan, C.	L257 (BM)		-	-	-
6088	Ramalinaceae	Malaysia	Borneo, Sabah, Danum valley	Thüs, Wolseley &Vairappan	D.8.07.1 (BORH)		**NONE**	-	-
1285	*Sporacestra* sp.	Malaysia	Borneo, Sa bah, Maliau Basin	Thüs, Wolseley &Vairappan	M.3.08.oQ.2 (BORH)		**NONE**	X	
5612	*Sporacestra* sp.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201033 (O)		**NONE**	**X**	**X2**
5614	*Sporacestra* sp.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201106 (O)		**NONE**	**X**	**X**
5617	*Sporacestra* sp.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201020 (O)		**NONE**	**XA, XR**	**X2**
5618	*Sporacestra* sp.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201048 (O)		**NONE**	**X**	**X**
5619	*Sporacestra* sp.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201101 (O)		**NONE**	**X**	**X**
5626	*Sporacestra* sp.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-201109 (O)		**NONE**	**XA***	**X2***
5631	*Sporacestra* sp.	Brazil	Pará, Thailândia, Fazenda Agroecológica São Roque	Dahl, Kistenich, Timdal & Toreskaas	L-202588 (O)		**FAT**	-	-
5632	*Sporacestra* sp.	Brazil	Pará, Thailândia, Fazenda Agroecológica São Roque	Dahl, Kistenich, Timdal & Toreskaas	L-202596 (O)		**NONE**	**XA**	**X**
5635	*Sporacestra* sp.	Brazil	Pará, Thailândia, Fazenda Agroecológica São Roque	Dahl, Kistenich, Timdal & Toreskaas	L-202591 (O)		**NONE**	-	**X***
6038	*Sporacestra* sp.	Malaysia	Borneo, Sabah, Kinabalu park	Paukov, A.	2231 (hb Paukov)		**NONE**	**X***	-
6039	*Sporacestra* sp.	Malaysia	Borneo, Sabah, Kinabalu park	Paukov, A.	2227 (hb Paukov)		**NONE**	**XB***	-
6046	*Sporacestra* sp.	Brazil	Pará, Thailândia, Fazenda Agroecológica São Roque	Dahl, Kistenich, Timdal & Toreskaas	L-202588 (O)		**FAT**	-	-
6081	*Sporacestra* sp.	Malaysia	Borneo, Sabah, Maliau Basin	Thüs, Wolseley &Vairappan	M.3.01.2 (BORH)		**NONE**	-	**X***
6082	*Sporacestra* sp.	Malaysia	Borneo, Sabah, Maliau Basin	Thüs, Wolseley &Vairappan	M.3.03.2 (BORH)		**NONE**	-	-
6083	*Sporacestra* sp.	Malaysia	Borneo, Sabah, Maliau Basin	Thüs, Wolseley &Vairappan	M132 (BORH)		**NONE**	**XB***	-
5621	*Sporacestra* sp.	Brazil	Pará, Melgaço, Floresta Nacional de Caxiuanã	Kistenich, S. & Timdal, E.	L-204486 (O)		**UNKN**	-	**X2***
5628	*Sporacestra* sp.	Brazil	São Paulo, Parque Estadual das Fontes do Ipiranga	Dahl, Kistenich, Timdal & Toreskaas	L-202768 (O)		**UNKN +TERP**	**X***	**X5*, XLR***
5634	*Sporacestra* sp.	Brazil	Pará, Thailândia, Fazenda Agroecológica São Roque	Dahl, Kistenich, Timdal & Toreskaas	L-202576 (O)		NONE	**X***	**X1***
-	*Toninia candida* (Weber) Th. Fr.	Norway		Bratli & Timdal	L-25779 (O)		-		SE59

2 Material and methods

2.1 Fieldwork

The fieldwork was conducted in tropical rainforests and tropical moist forests in Venezuela and Brazil during a period of 25 days in November – December 2015. I travelled with Einar Timdal, Sonja Kistenich, and Anne Karin Toreskaas, and we were accompanied by local researchers at all localities. The localities were chosen by the local researchers. An overview of the eight locations is presented in Table 2.

During fieldwork, the samples were preserved to prevent deterioration. We packed the collected specimens in acid free paper capsules and placed these between weighted wool cardboard in order to desiccate and flattened the specimens. After drying, the specimens were placed in sealed plastic bags with silica gel (as recommended by Chase & Hills, 1991).

The collections were made under local research and collection permits. The specimens collected in Venezuela were split in two at Universidad Central de Venezuela (VEN); the first set was retained in VEN and the second sent to the Natural History Museum in Oslo (O). The Brazilian collections were sent undivided to O, but will be split after completion of this study. The first set will be returned to Museu Paraense Emílio Goeldi (MG; the specimens from Pará) and Universidad de Federal de Sergipe (ISE; the specimens from São Paulo and Rio de Janeiro); the second set will be stored in O.

Table 2. An overview of field work locations in Venezuela and Brazil.

Country	Location	Habitat	Altitude
Venezuela	Miranda, Caracas, El Volcán	Carribean moist forest	1430-1480 m alt.
	Capital District, Parque Nacional Waraira Repano	Carribean moist forest	1800-2040 m alt.
	Capital District, Parque Nacional Macarao	Carribean moist forest	1860-2080 m alt.
	Carabobo, Parque Nacional Henry Pittier	Carribean rainforest	1150-1270 m alt.
Brazil	Parà, Tailândia, Fazenda Agroecológica Sâo Roque	Amazonian rainforest	50-60 m alt.
	Rio de Janeiro, Parque Nacional do Itatiaia	Atlantic rainforest	800-1200 m alt.
	São Paulo, Parque Estadual das Fontes do Ipiranga	Atlantic rainforest	790-830 m alt.
	São Paulo, Reserva Biológica do Alto da Serra de Paranapiacaba	Atlantic rainforest	790-810 m alt.

2.2 The specimens

The study is based on both newly collected specimens from Venezuela and Brazil and archived specimens from various herbaria. The fieldwork provided in total 29 newly collected specimens that were thought to belong in clade A, namely *Sporacestra, Bacidia* and *Bacidiopsora*. Due to the great morphological similarity of the genera, identification in field proved to be challenging. Ninety-six herbarium specimens suspected to belong in the same focus taxa (age up to 50 years) held at the following herbaria were also included: BM, BORH, BR, ISE, O, UPS, WIS, and the private herbaria of A. Frisch, A. Paukov, S. Pérez-Ortega and M. Seaward (herbarium abbreviations follow Holmgren et al. 1990). Eighteen sequences were borrowed from Kistenich et al. (in prep.; ongoing molecular phylogenetic studies of the Ramalinaceae). Twenty-six sequences of *Bacidia* were downloaded from GenBank, based on the taxon sampling in Ekman (2001). Available voucher data for all specimens are provided in Table 1.

2.3 DNA work

2.3.1 DNA-extraction

Up to 5 mg of tissue was sampled from 96 specimens, both freshly collected and herbarium specimens. If apothecia were present, they were sampled to ensure DNA originating from the mycobiont. The samples were placed into 2 mL microcentrifuge tubes with two sterilized 3 mm Tungsten-Carbide Beads (Applied Biosystems™, Foster City, California, U.S.A.), crushed using a Mixer Mill (MM301, Retsch GmbH & Co., Haan, Germany) and grinded for 2×1 min at 20Hz to ensure pulverized samples. I extracted DNA using the E.Z.N.A.® SP Plant DNA Kit (Omega Bio-Tek, Georgia, U.S.A) following the manufacturer's protocol, including the suggested additional elution step: samples were eluted twice (with 25 µL elution buffer in each) in two separate Safe-Lock Tubes™ (Eppendorf, Hamburg, Germany). Prior to final spinning, samples were incubated at 65 ºC for 5 min to increase DNA yield and concentration. The second eluates serve as back-up and are stored in the DNA collection at the Natural History Museum, University of Oslo.

2.3.2 Polymerase Chain Reaction (PCR)

I performed PCR amplification on all extracted DNAs to amplify the nuclear ribosomal internal transcribed spacer region (ITS: ITS1, 5.8S and ITS2) and the mitochondrial small subunit (mtSSU). Thus, including two markers from different genomes as proposed by Rubinoff and Holland (2005) . These DNA regions are commonly used in lichen systematics in general (e.g. Crespo & Lumbsch, 2010; Ekman, 2001; Lendemer et al., 2016; Schoch et al., 2012). Moreover, successful preliminary PCR amplification and sequencing of *Sporacestra* and relatives using internal primers for both the ITS (Bendiksby & Timdal, 2013) and mtSSU (Bendiksby, unpubl.) contributed to my selection of loci.

The ITS region was amplified in one single fragment using the primers ITS4/ITS5 (White et al., 1990). In case of no visible PCR product, the ITS1 and ITS2 regions were amplified in two separate reactions using the internal primers ITS-lichR and ITS-lichF (Bendiksby & Timdal, 2013; Table 3: ITS5 with ITS-lichR and ITS-lichF with ITS4). The first ca. 1000 base pairs (bp) of the mtSSU was amplified using the primers, mrSSU1 and mrSSU3R (Zoller et al., 1999). In case of no visible amplification, we amplified the region in two fragments (mtSSUA and mtSSUB, respectively) using the internal primers mtSSU-R and mtSSU-F with (Table 3; mtSSU1 with mtSSU-R, and mtSSU-F with mtSSU3R).

Table 3. List of primers used in the study with primer sequence and references.

DNA region	Primer name / primer sequence 5´→ 3´ direction	Reference
nrITS	ITS4 / TCCTCCGCTTATTGATATGC (rev)	White & al., 1990
	ITS5 / GGAAGTAAAAGTCGTAACAAGG (fwd)	White & al., 1990
	ITS-lichF / TGAATTGCAGAATTCAGTGAAT (fwd)	Bendiksby & Timdal 2013
	ITS-lichR / ATTCACTGAATTCTGCAATTCA (rev)	Bendiksby & Timdal 2013
mtSSU	mrSSU1 / AGCAGTGAGGAATATTGGTC (fwd)	Zoller & al., 1999
	mrSSU3R / ATGTGGCACGTCTATAGCCC (rev)	Zoller & al., 1999
	mtSSU-F / ACCAGTAGTGAAGTATGTTGTT (fwd)	Bendiksby (unpublished)
	mtSSU-R / AACAACATACTTCACTACTGGT (rev)	Bendiksby (unpublished)

Abbrevations: ITS = internal transcribed spacer; mtSSU = mitochondrial ribosomal small subunit; rev = reverse primer; fwd = forward primer.

The amplification of DNA was executede in 12 µL reactions using 0.1 µL AmpliTaq® DNA polymerase (Applied Biosystems™), 1.25 µL 10x buffer II, purified H_2O using Milli-Q® Integral Water Purification System (Millipore SAS, Molseim, France), 1.25 µL magnesium chloride (15 mM, $MgCl_2$, Applied Biosystems™), 0.25 µL dinucleotide triphosphate (10 mM, dNTP, Applied Biosystems™), 0.6 µL bovine serum albumen (1 g/L, BSA), 10 µM of each primer and 1.5 µL of the extracted DNA.

For some samples, Illustra™ puReTaq Ready-To-Go™ PCR Beads (GE Healthcare, Buckinghamshire, UK) were used following manufacturers protocol, except for diluting the content to double the amount of reactions.

The PCR amplifications were run on a 3×T100 Thermal Cycler (Bio-Rad Laboratories, Inc., California, U.S.A.) or a GeneAmp® PCR System 9700 (Applied Biosystems™) on the following cycle conditions: 95 ºC for 10 min, 34 cycles of 95 ºC for 30 s (mtSSU) or 45 s (ITS), 60 ºC for 30 s, 72 ºC of 30 s, followed by 72 ºC for 7 min and 4 ºC on infinite hold. All DNA isolates are deposited in the DNA collection at the Natural History Museum, University of Oslo.

2.3.3 Gel electrophoresis

Amplified PCR products were visualized with electrophoresis on 1% agarose gels, mixing SeaKem® LE Agarose (Lonza group, Basel, Switzerland) with 0.5×Tris-borate-EDTA-buffer. GelRed™ nucleic acid dye (Biotum, Hayward, California, U.S.A) was added to stain DNA. Each well was loaded with 4 µL PCR-product mixed with 4 µL homemade loadingbuffer (50 mM EDTA, 30% glycerol, 0.25% bromphenol blue and 0.2% xylene cyanol). One well per row was loaded with 3 µL Fastruler™ Low Range DNA Ladder (Fermentas® Thermo Fisher Scientific) for sequence length reference.

2.3.4 Cleaning PCR-products

PCR-products were cleaned using a 1:1 proportion of 2 µL of 10× diluted Exonuclease 1 (EXO I, New England Biolabs® inc, Massachusetts, U.S.A) and Shrimp Alkaline Phosphatase (rSAP, New England Biolabs® inc) to 6 µL PCR product. The mixture was incubated in the 3×T100 Thermal Cycler (Bio-Rad Laboratories, Inc.) at 37 ºC for 45 min, then at 80 ºC for 15 min.

2.3.5 Cycle sequencing

Cycle sequencing was done using a BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems™). The following master mix for very weak bands were used: 0.4 µL BigDye™ Terminator v3.1 Ready reaction mix (Applied Biosystems™), 1.8 µL 5× Sequencing Buffer (Applied Biosystems™), 6.3 µL MilliQ water, and 0.5 µL primer (10 µM). The mastermix was combined with 3.5 µL of cleaned PCR-product, giving a total of 10 µL. The cycle sequencing reaction was run on a 3×T100 Thermal Cycler (Bio-Rad Laboratories, Inc.) at 96 ºC for 1 min, 30 cycles of 96 ºC for 10 s, 50 ºC for 5 s, 60 ºC for 4 s, and then 4 ºC on infinite hold.

2.3.6 Ethanol precipitation

Each sequencing product was purified using 2 µL 1:1 mix of 0.125 M EDTA and 3M sodium acetate (NaAc), and 25 µL of 96% ethanol (EtOH). The samples were vortexed and centrifuged for 30 min at 4500 revolutions per minute (rpm) at 4 ºC with a plate centrifuge (Hettich Rotanta 46 RS Centrifuge, Andreas Hettich GmbH & Co. KG). The supernatant was removed by centrifuging the samples upside down for 15 s at 400 rpm. Then 35 µL of cold 70% EtOH was added and the samples centrifuged at 4500 for 25 s at 4 ºC. Another removal of the supernatant was executed by repeating the upside down centrifuge step (15 s at 400 rpm). The remaining liquid left in the tubes were removed by using the DNA120 SpeedVac vacuum centrifuge (Savant ™DNA SpeedVac™,Thermo Fisher Scientific, Massachusets, U.S.A), for 2 min on medium heat. The ethanol precipitated PCR products were stored at – 20 ºC.

2.3.7 DNA sequencing

Sequencing products were run on an ABI 3130×/ Genetic Analyzer (Applied Biosystems™). Eleven µL highly deionized formamide (Hi-Di) was added to the ethanol-precipitated products and left to rest for 15 minutes. Some of the sequencing was outsourced to Macrogen (Macrogen Europe, Amsterdam, The Netherlands). We used an Illustra™ ExoStar™ (GE Healthcare) 1-Step kit containing Illustra Alakaline phosphatase and Exonuclease 1, for cleaning the sequencing products, as recommended by Macrogen.

2.3.8 Sequence editing and alignment assembly

Sequences were assembled, thoroughly inspected by eye, and edited manually with Geneious 10.1 (Kearse et al., 2012). The datasets were preliminary aligned using the MAFFT online interface version 7 (Katoh et al., 2002; Katoh & Standley, 2013), with the multipair strategy. The default parameter settings were used, except for strategy which was set to –multipair (Accurate), and the scoring matrix for nucleotide sequences that was set to 20Pam / k = 2. Extensive manual adjustments were needed and undertaken in BioEdit (Hall, 1999). All sequences that possessed numerous indels and nucleotides in conflict with the rest of the dataset, indicating a distant relationship to the focus clade, were excluded.

2.3.9 Data assembly

Four different sequence alignments were assembled and analyzed: (1) A preliminary mtSSU alignment with 208 accessions that included a broad sampling of the Ramalinaceae (Kistenich et al., in prep, not shown in voucher table), all newly sequenced specimens, and *Bacidia* sequences from GenBank. The main goal of alignment 1 was to examine the phylogenetic positions and relationships of *Sporacestra*, *Bacidia* and *Bacidiopsora* to other members of the family. Sequence alignment 1 was important to guide in the species identification as well as in the selection of which new specimens to include in the study. Based on the preliminary Ramalinaceae phylogeny (Kistenich et al., in prep), I selected *Luckingia polyspora* for rooting in the phylogenetic analyses. (2) An mtSSU subset alignment that was formed by the analysis of alignment 1. It was assembled to examine the relationship within clade , containing 34 accessions that comprised the newly sequenced specimens, selected sequences borrowed from Kistenich et al. (in prep.), and *Bacidia* sequences from GenBank. *Luckingia polyspora* was used for rooting. (3) An ITS alignment with 42 accessions where mtSSU alignment 2 guided the assemblage. It comprised the newly sequenced specimens included in alignment 2, selected sequences that only successfully amplified the ITS region and belonging to the focus clade, sequences from Kistenich et al. (in prep.), and *Bacidia* sequences from GenBank. *Toninia candida* was used for rooting based on the sister relationship in the preliminary study of the Ramalinaceae (Kistenich et al., in prep). (4) A concatenation of alignment 2 and 3, containing 57 accessions.

2.4 Phylogenetic analyses and model selection

I analyzed the data using parsimony jackknifing (PJ) and Bayesian inference (BI) phylogenetic methods. I perform the PJ-analysis with 1000 replicates using WinClada (Nixon, 2002) through NONA (Goloboff, 1993). The sequence alignments were first run through SeqState (Müller, 2005) to analyze the number of parsimony- informative characters. To check for gene-tree incongruence, I compared the PJ consensus gene trees (i.e. sequence alignments 2 and 3).

The BI phylogenetic analyses were performed with MrBayes v.3.2.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). PartitionFinder (Guindon et al., 2010; Lanfear et al., 2012; Lanfear et al., 2016) were utilized to set the priors for the analysis. Settings were manually adjusted where branch lengths was set to linked, models of evolution only compatible for MrBayes were selected, and the Bayesian information criterion (BIC) was used for model selection. Data blocks were set for mtSSU, ITS1, 5.8S and ITS2. Schemes search was set to greedy. In MrBayes, the Markov Chain Monte Carlo (MCMC) was run with four chains (one cold and three heated chains) for 30 million generations, saving trees every 1000th generation. To check if the chains had converged, the average standard deviation of split frequencies (ASDSF) was controlled. The ASDSF should fall below 0.01. All the generations prior to the point of when the chains converged were discarded as burn-in, and the remaining trees were summarized as a 50% majority-rule consensus tree. The tree was visualized in FigTree (Rambaut, 2009), and edited with Microsoft® Powerpoint 2016. Only sequence alignment 4 was analyzed with Bayesian Inference.

2.5 Morphological investigations

Microscope investigations were carried out on microtome sections cut at 20-30 µm or on squash preparations. The preparations were observed in distilled water, 10% KOH (K), lactophenol cotton blue (LCB) and a modified Lugol's solution (I), where water was replaced by 50% lactic acid. Asci were studied in I after a few seconds pretreatment in K. Polarized light was used for locating crystals. The spore measurements are given as (x min) – $\bar{x}$ - SD – $\bar{x}$ – $\bar{x}$ + SD – (x max), where $\bar{x}$ is the mean length and SD the standard deviation.

2.6 Thin Layer Chromatography (TLC)

All specimens that showed to belonged in the genera of interest were subjected to thin-layer chromatography (TLC) using the standard methods of Culberson and Kristinsson (1970) and Culberson (1972), modified by Menlove (1974) and Culberson and Johnson (1982). All three solvent systems (A, B and C) were used to examine the lichen substances.

3 Results

3.1 Sequences and alignments

In total, 65 of the 96 DNA extracts rendered PCR products that were successfully sequenced, and several only for partial regions; 23 whole region sequences and 36 split region sequences of the mtSSU, 15 whole region and 21 split region sequences of the ITS. Sequences with low sequence quality, or that failed to show a relation within the focus clade after preliminary phylogenetic analysis of sequence alignment 1, were removed. Of 59 newly produced mtSSU sequences, 15 were included in the final analyses. Of 36 successful ITS sequences, 11 were included in the final analyses. The success rate was generally lower for older specimens of all species, and most of the DNA from the tropical specimens that were over 10 years of age did not amplify. Five mtSSU and 21 ITS sequences were downloaded from GenBank. The four analyzed sequence alignments (mtSSU alignment 1, mtSSU alignment 2, ITS alignment 3, and concatenated alignment 4) were 1527, 998, 610, and 1608 bp long, with 683, 259, 244, and 503 parsimony-informative characters (in-group, only), respectively.

PartitionFinder found three partitions in the concatenated sequence alignment 4 based on the four pre-determined data blocks (see results chapter above), namely mtSSU, ITS1 & ITS2, and 5.8s. The estimated best-fit nucleotide substitution models based on BIC were GTR+G for mtSSU, SYM+G for ITS1 & 2, and K80+1 for 5.8s.

3.2 Phylogenetic analyses

The preliminary parsimony jackknife analyses showed congruent gene trees from mtSSU alignment 2 and ITS alignment 3 (See Fig. S1: A and B), but resolved to various extents (ITS being the more resolved).

In the BI-analysis, the ASDSF had fallen to 0.0043 at termination (30 million generations), and the first 7500 trees (25%) were discarded as burn-in, removing all trees with an ASDSF > 0.01 as recommended in the MrBayes manual. The remaining trees were summarized into a Bayesian 50% majority-rule consensus tree, presented in Fig. 2. The preliminary PJ phylogeny is mainly consistent with the BI phylogeny (see Fig. S2), with the

exception of a branch collapse of the 1433 *Phyllopsora sorediata* accession, in the BI-tree (Fig. 2: Clade C).

The phylogenetic result (Fig. 2.) presents a well-supported topology that is highly resolved. The branch support (PP/JK) is of varying degree, with the terminal branches having higher support than the backbone. From the phylogenetic results, we can see that clade A has received high branch support from the molecular analyses (1/≤ 85, PP/JK). Sister to clade A is a not supported group of *Bacidia.* Clade A splits into two well-supported subclades (both 1/ ≤ 85), hereby referred to as clade B and clade C.

Clade B includes 12 accessions of members tentatively determined to *Phyllopsora*, *Physcidia* and *Sporacestra*. Basal in this clade, we find 395 *Physcidia* sp. as a highly supported (1/≤ 85) sister to what is hereby called clade B'. Clade B' contains 11 accessions of *Sporacestra* and *Phyllopsora*, with high support (1/≤ 85). Both *Sporacestra* and *Phyllopsora* are paraphyletic, based on the topology in clade B'. However, this is merely an artifact of preliminary naming of the collections during fieldwork, where new collections have been named *Sporacestra* sp. rather than *Phyllopsora* sp.

Clade C includes 31 accessions of species currently included in *Bacidia*, *Bacidiopsora* and *Phyllopsora*. Three accessions from Thailand, tentatively determined by Einar Timdal as *Bacidiopsora* sp. 1, are the most basal group in clade B with high branch support (≤ 0.95/71). *Bacidiopsora* sp. 1 did not group together with the other members of *Bacidiopsora*, making the genus polyphyletic.

Three accessions of *Phyllopsora sorediata* from Thailand group together with *Bacidia laurocerasi* ssp. *laurocerasi* and *Bacidia biatorina*, but with low support (Fig. 2: clade C). In the PJ phylogeny, *P. sorediata* group together with higher support (JK = 75; See Fig. 2: clade C; Fig. S2: clade C). The topological placement of *P. sorediata* separated from *P. borbonica* and *P. pertexta* (Fig. 2: clade B'), makes *Phyllopsora* polyphyletic in clade A.

As indicated above, *Bacidia* is polyphyletic and splits into two subclades. One of the subclades lumps into clade C, and includes the type species of *Bacidia* (*B. rosella*). Nested into *Bacidia* in clade C, we find the *Bacidiopsora* group, highly supported as monophyletic (1/81). This monophyletic group includes eight accessions, both Neotropical (Venezuela and

Ecuador) and Pantropical (South Africa). See the discussion for more detailed information about the phylogeny.

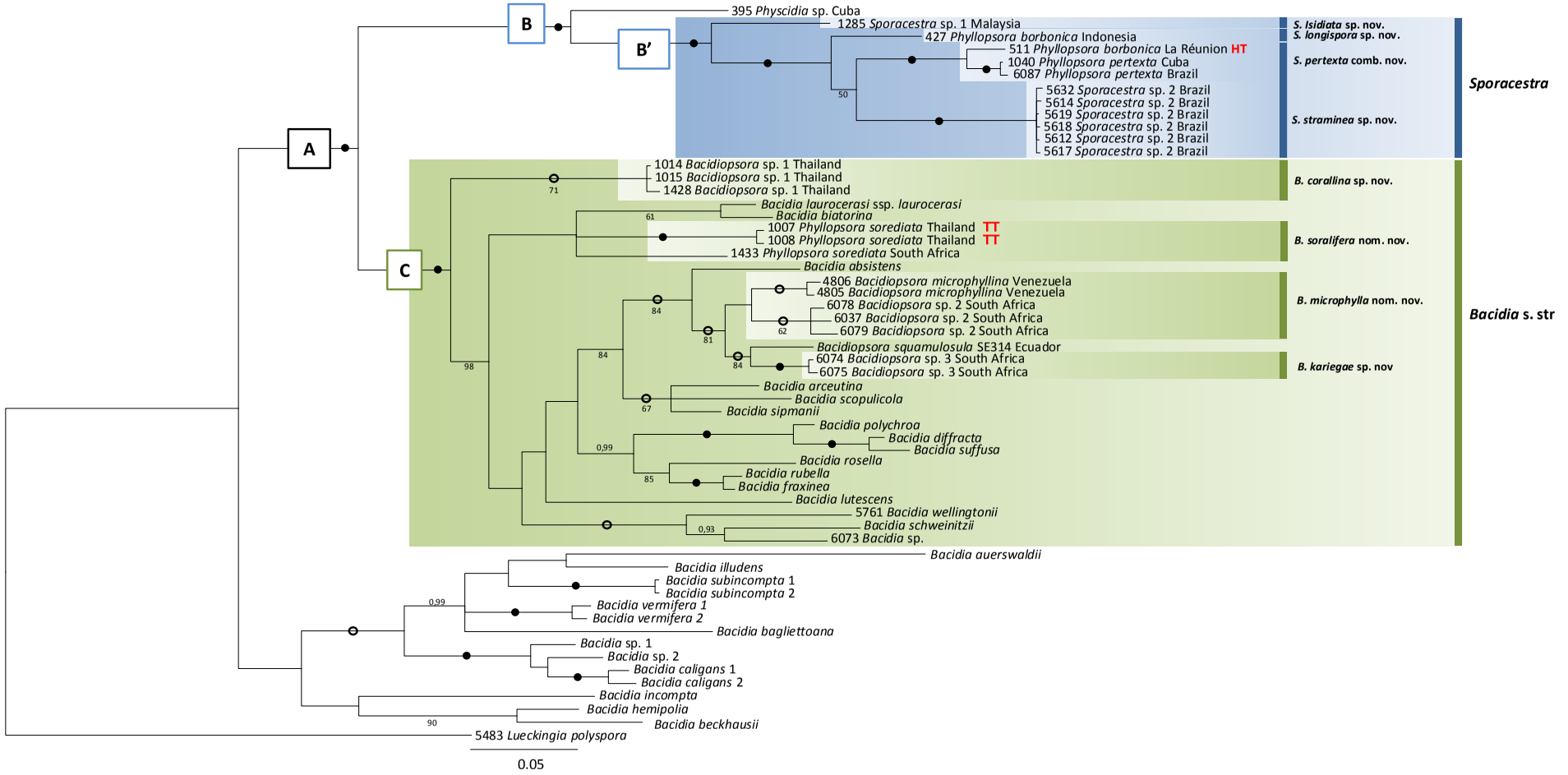

Fig. 2: The Bayesian 50 % majority rule consensus phylogram based on 57 accessions and a 1608 basepair long alignment, from one nuclear (ITS) and one mitochondrial (SSU) marker. It consits of accessions from *Bacidia*, *Bacidiopsora*, *Phyllopsora* and *Sporacestra*. *Lueckingia polyspora* is chosen as outgroup. Posterior probabilities (PP) is reported above branches and parsimony jackknife (JK) branch support underneath. Support values that are below 0.9/50 (PP/JK) are not reported. ● indicates 1 ≤ 85 node support, ○ indikates PP ≤ 0.95. Species names to the right of the coloured bars indicate the classification as support herein. Letters to the left in the backbone represents clades introduced in the Results chapter (clade A, clade B, clade B' and clade C). HT = holotype, TT = «topotype» (same locality, same date as holotype). Branch lengths corresponds to number of substitutions per site, assuming the following substition models: GTR+G for mtSSU, SYM+G for ITS1 & ITS2 and K80+1 for 5.8s. Bar represents 0.05 % change.

3.3 Morphological investigations

Most of the *Bacidia* accessions were borrowed from GenBank, and no morphological or anatomical investigations have been carried out on those specimens. Comparing the morphology of the specimens in clade B (Fig. 2) clearly showed that the morphology of *Physcidia* sp. differed from clade B' by having a subfoliose thallus. The single collection of this species was sterile, thus unsuitable for investigations on apothecial anatomy. Clade B' contained five phenotypes, based on 26 specimens. All members shared the character of having a well-developed brownish black prothallus with granules forming the thallus and long non-septate ascospores, and differed mainly in the morphology of the apothecia. Investigations on *Bacidiopsora* sp. 1 and the *Bacidiopsora* group in clade C was based on four and eight specimens, respectively. They both shared the character of having a dark prothallus and acicular, distinctly septate ascospores. They differed in that the *Bacidiopsora* sp. 1 had a granulose thallus with coralloid isidia, whereas the *Bacidiopsora* group had a squamulose thallus. Also, *Bacidiopsora* sp. 1 contained crystals in the excipulum only, while the *Bacidiopsora* group contained crystals in the hypothecium as well. The *Bacidiopsora* subgroup contained three phenotypes, differing in the presence of apothecia and the shape of the squamules. The morphological investigations on *Phyllopsora sorediata* were based on seven specimens and showed that the species possess a white to grey prothallus with an areolate or subsquamulose thallus forming soralia. Further results from the morphological investigation are elaborated in the Discussion chapter and presented in the Taxonomy chapter below.

3.4 TLC

The TLC results revealed that eight lichen substances were present in clade A (Fig. 2). Most of *Bacidia* was borrowed from GenBank, and no chemical investigations have been carried out on those specimens. The specimens in clade B' showed no trace of any lichen substances; in contrast to the sister accession 395 *Physcidia* sp. which contained atranorin, zeorin and two unknown compounds. All *Phyllopsora sorediata* specimens contained both atranorin and divaricatic acid. Specimens in *Bacidiopsora* sp. 1 and the *B. squamulosula* group both showed major occurrences of hyperhomosekikaic acid and/or homosekikaic acid. I was unable to clearly distinguish between the two substances, although the former has a slightly higher R_f value in solvent system B. These will hereafter be referred to as hyperhomosekikaic acid

and/or homosekikaic acid. *Bacidiopsora* sp.1 also contained an unidentified xanthone. An overview over the lichen substances detected in the focal group is listed in Table 4.

Table 4: *Lichen substances detected in the focal group.*

Extr. Species/chemistry	none	atr	div	hhsek/hsek	unk1	unk2	xan	zeo	fat
395 *Physcidia* sp.	·	.	M	.	M	M	·	M	·
1285 *Sporacestra* sp. 1	x	.	.	.	.	.	·	.	·
427 *Phyllopsora pertexta*	x	.	.	.	.	.	·	.	·
511 *Phyllopsora borbonica*	x	.	.	.	.	.	·	.	·
1040 *Phyllopsora pertexta*	x	.	.	.	.	.	·	.	·
6087 *Phyllopsora pertexta*	x	.	.	.	.	.	·	.	·
5632 *Sporacestra* sp. 2	x	.	.	.	.	.	·	.	·
5614 *Sporacestra* sp. 2	x	.	.	.	.	.	·	.	·
5619 *Sporacestra* sp. 2	x	.	.	.	.	.	·	.	·
5612 *Sporacestra* sp. 2	x	.	.	.	.	.	·	.	·
5617 *Sporacestra* sp. 2	x	.	.	.	.	.	·	.	·
5618 *Sporacestra* sp. 2	x	.	.	.	.	.	·	.	·
1014 *Bacidiopsora* sp. 1	·	.	.	M	.	.	M	.	·
1015 *Bacidiopsora* sp. 1	·	.	.	M	.	.	M	.	·
1428 *Bacidiopsora* sp. 1	·	.	.	M	.	.	M	.	·
1007 *Phyllopsora sorediata*	·	M	M	.	.	.	·	.	·
1008 *Phyllopsora sorediata*	·	M	M	.	.	.	·	.	·
1428 *Phyllopsora sorediata*	·	M	M	.	.	.	·	.	·
4806 *Bacidiopsora microphyllina*	·	.	.	M	.	.	·	.	·
4805 *Bacidiopsora microphyllina*	·	.	.	M	.	.	·	.	·
6078 *Bacidiospora* sp. 2	·	.	.	M	.	.	·	.	·
6079 *Bacidiospora* sp. 2	·	.	.	M	.	.	·	.	·
6037 *Bacidiospora* sp. 2	·	.	.	M	.	.	·	.	·
6074 *Bacidiopsora* sp. 3	·	.	.	M	.	.	·	.	·
6075 *Bacidiopsora* sp. 3	·	.	.	M	.	.	·	.	·
508 *Bacidiopsora squamulosula*	·	.	.	M	.	.	·	.	·

Abbrevations: Extr. = DNA extraction number, atr = atranorin, div = divaricatic acid, hhsek/hsek = hyperhomosekikaic acid/homosekikaic acid, unkn = unknown lichen substance, xan = unidentified xanthone, zeo = zeorin, fat = fatty acid. M = Major occurrence.

4 Discussion

In this study, I assess the phylogenetic relationships in a poorly known group of tropical lichens, namely clade A of the Ramalinaceae; see Fig. 1. Although the large genus *Bacidia* has been extensively studied using both traditional and molecular methods (Czarnota & Coppins, 2007; Ekman & Nordin, 1993; Lendemer et al., 2016; Llop & Gómez-Bolea, 1999), there are only very few studies on the much smaller and presumably closely related genus *Bacidiopsora* (A. Aptroot, 2002; Kalb, 1988, 2004; Kalb & Elix, 1995), of which none include DNA sequence data. The possible resurrection of the genus *Sporacestra* as stated by Ekman (1996) has been largely overlooked in the past (e.g. Lumbsch & Huhndorf, 2010; Lücking et al., 2016), and no DNA sequences exist in GenBank under this or synonymous names. Thus, this is the first molecular systematic study that includes members of the genera *Bacidiopsora*, *Phyllopsora* and *Sporacestra*. The aim has been to examine the following hypotheses: (1) *Sporacestra* represents a monophyletic genus; (2) *Phyllopsora borbonica* is synonymous with *P. pertexta*; (3) *Bacidiopsora* sp. 1 represents a new genus; (4) *Phyllopsora* sorediata represents a new genus; (5) *Bacidiopsora* is monophyletic, and; (6) *Bacidiopsora* is not nested within *Bacidia*.

A large component of the biodiversity of lichenized fungi is overlooked and underestimated partly due to the inconspicuous nature of small crustose lichens (Hodkinson & Lendemer, 2012). Many groups of lichenized fungi are taxonomically challenging due to few informative phenotypic characters and a high level of homoplasy (Buschbom & Mueller, 2004; Gueidan et al., 2007; Pino-Bodas et al., 2011). An example of this is the usage of thallus architecture as a guide for delimiting lichen genera (Muggia et al., 2011), as exemplified by the typical *Phyllopsora* growth form, i.e. having a squamulose thallus on a tightly woven prothallus. This character seems to have evolved multiple times in the tropical habitat in the Ramalinaceae, being present in at least *Bacidiopsora*, *Eschatogonia*, *Krogia*, *Phyllopsora*, and *Physcidia* (E. Timdal, pers. comm.). Moreover, since the current knowledge of the phenotypic variation in the focal genera is poor, my preliminary species determination in the field proved to be challenging. Therefore, using an integrative approach to taxonomy as recommended by e.g. Dayrat (2005) and Lumbsch & Leavitt (2011) , has been crucial in this study.

My phylogenetic results, based on two DNA markers and an expanded taxon sampling, corroborates a highly supported clade in the Ramalinaceae that consists of several species currently included in *Bacidia, Bacidiopsora, Phyllopsora,* and *Sporacestra* (Fig. 2: clade A). The phylogenetic hypothesis is generally well supported, both in the deeper (the oldest speciation events) but mostly in more terminal parts (recent speciation events; Fig. 2). The Bayesian majority-rule consensus topology (Fig. 2: clade A) corroborates the preliminary unpublished study of Bendiksby & Timdal (Fig. 1: clade A). Moreover, the status of *Bacidia* as being polyphyletic also corroborates Ekman (2001), where largely the same species of *Bacidia* were included. In the following section, I will discuss the members of clade B' and clade C (Fig. 2) in light of morphological (including anatomical), chemical, and molecular results. Further, I will also propose taxonomic changes when there is support from multiple sources of evidence.

4.1 Clade B': *Sporacestra* and *Phyllopsora*

The molecular phylogeny shows that there is a clade, namely clade B' in Fig. 2, that is highly supported (1/≤85). The clade comprises species preliminary designated to a resurrected *Sporacestra,* together with four accessions of *P. pertexta* and *P. borbonica*. The circumscription of *Phyllopsora* is under revision (Kistenich et al., in prep.), and the core of the genus, including the type species, *P. breviuscula,* belongs outside clade A (Bendiksby & Timdal, unpubl.; Fig 1). The apparent paraphyly of both genera in clade B' is, as earlier mentioned, merely an artefact of preliminary naming. As already stated, *P. pertexta* is synonymous with the type species of *Sporacestra* (*Biatora prasina*), this being the basis of the resurrection of *Sporacestra.* Thus, hypothesis (1) *Sporacestra* is monophyletic, can be accepted based on this phylogeny.

It became apparent during the fieldwork that separating what might represent members of *Sporacestra* from some members of *Phyllopsora* was almost impossible. Based on the 11 accessions representing clade B', with 15 additional specimens, the characters delimitating this genus was investigated. The results showed that the main delimiting character separating *Sporacestra* from *Bacidia, Bacidiopsora,* and *Phyllopsora* is the morphology of the ascospores, a character impossible to use in the field without microscopy. In more detail, *Sporacestra* differs from *Bacidia* and *Bacidiopsora* in having long, acicular, non-septate to pseudoseptate ascospores. Moreover, the genus differs from *Bacidiopsora* by not containing

any lichen substances. *Sporacestra* is morphologically similar to *Phyllopsora*, and the only reliable evidence for separating members in the two genera appears to be with molecular analyses. The genus *Aciculopsora*, described from Costa Rica (Aptroot et al., 2006), is also morphologically similar to *Sporacestra* in having a crustose thallus and acicular ascospores. This genus, however, belongs outside clade A (S. Kistenich, pers. comm.).

Basal in clade B' we find *Sporacestra* sp. 1 (Fig. 2) as a highly supported sister to the other accessions in clade B'. It is apparent from the phylogeny that this species is genetically separated from the other members of *Sporacestra*, illustrated with a long branch. Morphological investigations on the sequenced and four additional specimens revealed that this species is separated from the other members of clade B' by the presence of isidia. With both the molecular and morphological evidence, I decide to describe this as a new species, *S. isidiata* to be included in the resurrected *Sporacestra* (see the Taxonomy chapter below)

Further in clade B', we can see from the phylogeny that *P. borbonica* is represented by two accessions (427 and 511, the holotype) that are genetically separated. The accession 427 *P. borbonica* is a sister to 511 *P. borbonica*, *P. pertexta* and *Sporacestra.* sp. 2, indicating that it may represent an undescribed species. Alternatively, it might indicate geographic distance and lack of gene flow. Results of a sequence identity matrix between 427 *P. borbonica* and the accessions of 511 *P. borbonica* revealed that the similarity was at 50%. Closer morphological (including anatomy) examinations on the sequenced and 11 additional specimens affirmed distinct differences, including dull yellow, plane to concave apothecia in 427 *P. borbonica*, in contrast to brown, plane to convex apothecia in *P. borbonica*. On the basis of both the molecular data, combined with the morphological, I therefore conclude that 427 *P. borbonica* constitutes a new undescribed species, *S. longispora*, to be described in the Taxonomy chapter below.

Nested into clade B', we find a subclade with *P. pertexta* and *P. borbonica. Phyllopsora pertexta* was described from the Neotropics (Cuba) some 150 years ago (Nylander, 1863), while *P. borbonica* was described much later from the Pantropics (La Réunion and Mauritius; Timdal & Krog, 2001). Timdal & Krog (2001) did not discuss or compare *P. borbonica* with *P. pertexta*. Later, however, Timdal (2011) commented on the possible synonymy of the two species, and indicated that the color of the hypothecium is the only character distinguishing them. In this study, I was not able to separate the two species in the coloration of the hypothecium, nor in any other character. Comparing the DNA sequence of the two species, a

sequence identity matrix revealed that the sequences are 97% similar, compared to the similarity of *P. borbonica* to the other sequences in clade B', which all fell under 50%. The split between 511 *P. borbonica* (the holotype) from the two accessions of *P. pertexta* (from Cuba and Brazil) could be due to the fact that the accession is only represented by mtSSUA and ITS2. Another explanation for the split could be due to the geographical distance between the accessions, 511 *P. borbonica* being Pantropical, where the two accessions of *P. pertexta* are Neotropical. On the basis of this, it would not be appropriate to keep them described as two separate species. I therefore consider *P. borbonica* and *P. pertexta* to be synonymous and the new combination *S. pertexta* is formally made in the Taxonomy chapter below. Moreover, with this I accept hypothesis (2) *P. pertexta* is synonymous with *P. borbonica*.

Sister to *S. pertexta* in clade B' (Fig. 2) is a subclade that consists of six accessions of *Sporacestra* sp. 2 from two different localities in Brazil (see Table 1). The accessions are highly homogeneous molecularly, and the genetic distinctness and internal homogeneity of this group support this as a good species. Alternatively, this could represent a new genus, based on the high degree of genetic divergence portrayed by the long branch. However, since this clade share most characters with the rest of clade B' (Fig. 2: clade B'), as the character of having long acicular spores, there is no basis of describing this as a new genus, when clade B' strongly appears as a natural group. The subclade is distinguished from most species currently included in *Sporacestra* by the plane to convex, dull yellow apothecia. On account of the molecular, morphological, and anatomical results, I regard this as a new species to be described as *S. straminea* (see the Taxonomy chapter below).

The molecular data, combined with morphology (including anatomy) and chemistry, revealed that the 11 accessions in the phylogeny (Fig. 2: clade B') represents four species to be included in a monophyletic, resurrected *Sporacestra*. Clade B' is highly supported in every internal branch, except for a lack of support in the *S. pertexta* and *S. straminea* group (>0.9/50). As both the *S. pertexta* and *S. straminea* clade receive high support, this might indicate a lack of taxon sampling and more undescribed species may belong in this clade. On the other hand, an alternative polytomy with *S. longispora*, *S. pertexta* and *S. straminea* may represent three closely evolving species and a further support on the monophyly of *Sporacestra*.

4.2 Clade C: *Bacidia*, *Bacidiopsora* and *Phyllopsora*

My phylogenetic result highly supports clade C (Fig. 2; 1/≤85), consisting of *Bacidia*, *Phyllopsora sorediata* and members currently included in *Bacidiopsora* as a sister to clade B. *Bacidia* is a large genus with high morphological and anatomical variation. The generic description I follow was made by Ekman (1996) on *Bacidia* species from North America, and there has never been made a modern revision of all *Bacidia* species worldwide. In this phylogeny (Fig. 2) we can see that *Bacidia* is polyphyletic with its type species (*Bacidia rubella*) present together with other members in clade C. Many members of *Bacidia* fall outside clade A. The same is shown in Ekman (2001), where he defines the species present in this clade C as *Bacidia* sensu stricto, a definition I hereby adopt. Several members of *Bacidia* sensu lato has recently been studied and moved to other genera (Kalb et al., 2000; Lücking et al., 2001).

Basal in clade C, we find three accessions tentatively determined as *Bacidiopsora* by Timdal (1014, 1015 and 1428 *Bacidiopsora* sp. 1 from Thailand, see Fig. 2) with high PP support (=1). The determination was based upon the presence of a dark prothallus, a squamulose thallus, pluriseptate ascospores, and the content of hyperhomosekikaic and/or homosekikaic acid. The molecular phylogenetic result tells a different story, placing *Bacidiopsora* sp. 1 outside the rest of *Bacidiopsora*, indicating that these *Bacidiopsora* type characters are a product of convergent evolution (cfr. Muggia et al., 2011).

The three accessions of *P. sorediata* (1007, 1008 and 1433, all "topotypes" = collected on the same date, same locality as holotype: Fig. 2: clade C) are not supported as monophyletic, and there seems to be a geographic component to it; the two accessions from Thailand form a supported group, whereas 1433 from South Africa falls out in a polytomy that also includes a clade with *Bacidia laurocerasi* ssp. *laurocerasi* and *Bacidia biatorina*. The branch collapse of 1428 *P.sorediata* in the BI-phylogeny (See fig. 2; Appendix Fig. S2) is probably due to the fact that the accession is only represented by mtSSUB. This species were originally described and placed in *Triclinum* by Aptroot (2007), based on the prothallus, squamulose thallus and the presence of atranorin, and divaricatic acid. Timdal (2011) transferred it to *Phyllopsora* on the basis of his synonymization of *Triclinum* with *Phyllopsora*. The preliminary phylogeny (Fig. 1; Bendiksby & Timdal, unpubl.) shows that *P. sorediata* does not belong in

Phyllopsora. Ongoing studies by Kistenich and co-workers show that it does not belong in *Triclinum* either (S. Kistenich, pers. comm.).

My DNA sequence phylogeny places a second clade of *Bacidiopsora* accessions (the one including the type species of the genus, *B. squamulosula*) nested within *Bacidia* s. str., thus rejecting hypothesis (5) *Bacidiopsora* is monophyletic and (6) *Bacidiopsora* is not nested into *Bacidia*. In addition to the type species, the *Bacidiopsora* clade appear to hold three distinct genetic lineages (Fig. 2: clade C, *B. microphyllina*, sp. 2, and sp. 3), which gains support also from morphological and anatomical investigations. Three accessions (6037, 6078 and 6079), referred to as *Bacidiopsora* sp. 2, has the same morphology and anatomy as *B. microphyllina,* and I conclude that they represent the same species. The split between *Bacidiopsora microphyllina* and sp. 2 in the phylogeny could be explained by the geographical distance and hence lack of gene flow between the populations, the former being Neotropical (Venezuela), the latter Pantropical (South Africa). The sister clade to *Bacidopsora microphyllina* (incl. sp. 2) consists of *Bacidiopsora squamulosula* and two accessions (6074 and 6075) referred to as *Bacidiopsora* sp. 3. The latter species differs from all other species currently included in *Bacidiopsora* by having squamules dissolving into granular soredia (Fig. 5, Taxonomy chapter below). The taxon sampling of *Bacidiopsora* is still not satisfying, as my attempts to sequence *B. orizabana*, *B. psorina*, *B. silvicola,* and *B. tenuisecta*, all failed. Most of the specimens (except *B. tenuisecta* from BR) were borrowed from herbarium Klaus Kalb, now in WIS, that was collected in the tropics from 1980–2002. The collections may therefore be too old for molecular work, due to degradation of DNA (cfr. Gueidan et al., 2016). Alternatively, it could be that the methods used for DNA extraction in this study are not well-suited for old specimens and low DNA concentration, and an adjustment of the extraction procedure may be needed.

As I see it, there are two alternatives for the taxonomic treatment of *Bacidiopsora* sp. 1, *Bacidiopsora*, and *P. sorediata.* (1) Lumping the three groups into *Bacidia* s. str., thus making clade C one big, anatomically and morphologically variable, but monophyletic genus. In that case, the concept of the genus *Bacidia* s. str. will be extended to include species with a well-developed squamulose thallus, pseudoseptate ascospores, and with the secondary compounds divaricatic acid, hyperhomosekikaic acid, and homosekikaic acid. (2) Splitting the clade, putting the strongest weight on morphology, describing *Bacidiopsora* sp. 1 and *P. sorediata* as to two new genera. Thus, keeping a monophyletic *Bacidiopsora* within clade C, making

Bacidia s.str. paraphyletic. However, as discussed earlier in this chapter, thallus morphology may not be a good character at the genus level, merely a rather easily evolving adaption to ecological conditions (Gueidan et al., 2007). Moreover, the presence or absence of lichen substances is due to the expression of the genotype, and may be a product of the lichen's ecological adaption (Brodo, 1986). This can be seen in the sekikaic acid-complex, which is widely distributed throughout the Ramalinaceae, present in *Bacidiopsora, Eschatogonia, Phyllopsora, Physcidia,* and *Ramalina* (Kalb, 2004; Krog & James, 1977; Swinscow & Krog, 1988; Timdal, 2008). This shows that the secondary chemistry may not be a good delimiting character at the genus level. Now, with the addition of molecular data to infer on the evolutionary relationship, this should be treated as first priority in delimiting genera (Lumbsch & Leavitt, 2011). Choosing alternative (2) could make the taxonomy complicated, and may be misleading about character distribution and evolution. Furthermore, I believe that it would be premature to split the clades when *Bacidia* s. lat. is in such an unstable state regarding its circumscription, due to the lack of molecular data for the majority of the species. Therefore, I conclude with taxonomical alternative (1), which is to lump *Bacidiopsora* sp. 1, *Bacidiopsora,* and *P. sorediata* into *Bacidia* s. str., which in turn leads to rejection of both hypotheses (3) and (4), i.e. that *Bacidiopsora* sp. 1 and *P. sorediata* represents two new genera. Moreover, I will describe *Bacidiopsora* sp. 1 and *Bacidiopsora* sp. 3 as two new species to be included in *Bacidia,* namely, *Bacidia corallina* and *Bacidia kariegae.* New names are needed for *B. microphyllina* and *P. sorediata* as the epithets are already used in *Bacidia* (see the Taxonomy chapter, below).

4.3 Conclusion and final remarks

This study investigated the phylogenetic relationships within the tropical genera *Bacidia, Bacidiopsora* and *Sporacestra* (clade A) in the family Ramalinaceae. It proved to be challenging to obtain quality DNA sequence data from this group of tropical lichens. The quality of the molecular data was thoroughly gone through by manually editing and excluding sequences with low quality, to ensure the best results from the analyses. The results in this thesis show that many phenotypic characters in the Ramalinaceae are a product of convergent evolution, and this emphasizes the importance of molecular systematics. The integrative approach, combining traditional taxonomy (morphology, anatomy, and chemistry) and molecular methods, led me to the conclusions that *Sporacestra* is a monophyletic genus and

that all species in clade C should be lumped into *Bacidia* s. str. A consequence of this is that I describe five new species, synonymize one species name (*Phyllopsora borbonica)*, synonymize one genus name (*Bacidiopsora*), resurrect one genus name (*Sporacestra*), make one new combination, and two new names in *Bacidia* s. str. (see the Taxonomy chapter, below).

During this two-year period of working with the thesis, several complications arose and became clear along the way. First of all, working with in the tropical habitat is in itself a challenging task, due to the warm, humid conditions in the dense vegetation. To be able to perform such a task, you are completely dependent on local researchers, not only to guide and plan the logistics, but also to obtain research permits. International agreements, e.g. the Nagoya protocol, has made it challenging to collect outside Norway, especially in Brazil, where everything collected has to be registered in a local herbarium and from there sent to Norway as duplicates or loans. This procedure can often take up to several months, and it does not help that genomic DNA of crustose, corticolous lichens seems to degrade quite fast after specimen desiccation (Gueidan et al., 2016). Moreover, species (even genus) identification in the field may also be difficult, especially when the genera in question have few diagnostic characters. Most of my fieldwork collections were excluded from the dataset after preliminary analysis of sequence alignment 1 (Table 1, indicated with *), because they fell outside clade A or the sequence quality was too low to be included.

4.4 Further studies

This study is only scratching the surface of the undescribed species diversity in the Tropics. If I had the opportunity to prolong this study, I would have gone back in the laboratory optimizing procedures with the aim to obtain both genetic markers for all included accessions. Moreover, DNA sequences of the four remaining species of *Bacidiopsora* (*B. orizabana, B. psorina, B. silvicola*, and *B. tenuisecta*) have to be successfully obtained and placed in a molecular phylogeny before further conclusions can be made. Future studies should also include additional genetic markers, e.g. the protein-coding, nuclear RPB1 and MCM7 markers, to increase the chance of the phylogeny to reflect the true evolutionary history and relationship of these clades. These markers have been shown to be useful in species delimitation on lichens (e.g. Divakar et al., 2017; Schmitt et al., 2012). I would also recommend trying the procedure of using FTA cards on freshly collected tropical specimens,

as recommended by Gueidan et al. (2016). In this procedure, DNA from tropical collections is extracted directly from specimens in the field (by the use of Polybutylene terephthalate [PBT]) and stored on FTA cards, which bind and preserves the DNA. Lastly, and most importantly, *Bacidia, Bacidiopsora* and *Sporacestra* should be placed into a Ramalinaceae phylogeny, to ensure broad and extensive taxon sampling. This can provide further understanding of these rather inconspicuous tropical species.

5 Taxonomy

Sporacestra A. Massal.

in Atti Reale Ist. Veneto Sci. Lett. Arti., ser 3, 5: 264 (1860). Type: *Biatora prasina* Mont. & Tuck. [non (Fr.) Fr.] [= *Sporacestra pertexta*].

Prothallus present, white when young, becoming brownish black. Thallus crustose, granulose, containing unicellular green algae; cortex one or two cell layers thick, composed of rather thin-walled hyphae with rounded lumina. — Apothecia biatorine, yellow to brown; proper excipulum colorless to pale brown in inner part, colorless to dark brown in the rim, composed of radiating, conglutinated, rather thick-walled hyphae with shortly cylindrical lumina; hypothecium not distinctly delimited from exipulum, colorless to reddish or dark brown, chondroid, composed of irregularly oriented, thick-walled hyphae with cylindrical lumina; hymenium colorless, with amyloid gelatin, 70–90 µm high; paraphyses conglutinated, straight, simple or rarely branched, c. 5 µm wide, with a slightly swollen, colorless or faintly brown, apical cell; ascus narrowly clavate, with a well-developed, amyloid tholus usually with an ocular chamber; ascospores acicular, straight, parallel in the ascus, simple or irregularly pseudoseptate, colorless. — Pycnidia unknown. Chemistry: No lichen substances (by TLC).

Key to the species of *Sporacestra*

1	Thallus with simple, pale brownish green, cylindrical isidia, patchily distributed on thallus.	***S. isidiata***
	Thallus without isidia	**2**
2(1)	Apothecia colour reddish to purple brown	***S. pertexta***
	Apothecia colour dull yellow	**3**
3(2)	Apothecia plane to concave, ascospore mean length < 55 µm	***S. longispora***
	Apothecia plane to convex, ascospore mean length > 55 µm	***S. straminea***

Sporacestra isidiata Stapnes & Timdal, sp. nov. [MB XXXXX]

Diagnosis: Prothallus thick, brownish black. Thallus with isidia patchily distributed on the thallus; isidia cylindrical, simple, c. 0.06 wide and 0.6mm long, pale brownish green.

Typus: Malaysia, Malaysian Borneo, Sabah, Maliau Basin, 4.7476°N, 116.9675°E, pristine lowland dipterocarp forest, 2012, *P. Wolseley, H. Thüs, & C. Vairappan M.3.08.oQ.2* (BORH, holotypus! [TLC: No lichen substances detected; GenBank: XXXXXXXX]).

(Fig. 3A)

Prothallus thick, white when young, soon becoming brownish black. Thallus effuse, crustose, formed by small (up to 0.1 mm wide), adnate, isodiametric granules which are discrete to adjoined peripherally and soon forming a more or less continuous crust; granules pale green, glabrous, pubescent along the margin; isidia present, often patchily distributed, originating one per granule, cylindrical, c. 0.06 mm wide, up to 0.6 mm long, simple, pale brownish green, glabrous; upper cortex composed of 1–2 layers of thin walled hyphae with rounded cells, 5–10 µm thick; algal layer filling the inner part of the granules; cortex and algal layer not containing crystals. — Apothecia and pycnidia not seen.

Distribution and ecology. Malaysian Borneo in the Maliau Basin. Crustose on tree bark in pristine lowland dipterocarp forest.

Notes. Sporacestra isidiata differs from all other species in *Sporacestra* by the presence of isidia. Apothecia have not been seen in this species. The organization and morphology of the external and internal structures is similar to the other species included in the genus.

Etymology. *Isidata*, named after the presence of isidia.

Additional species examined: **Malaysia:** Malaysian Borneo, Sabah, Maliau Basin, 4.7476°N, 116. 9676°E, pristine lowland dipterocarp forest, 2012, *P. Wolseley, H. Thüs & C. Vairappan, M-132, M3-0I-2, & M3-03-2*, (all BORH).

Sporacestra longispora Stapnes, sp. nov. [MB XXXXX]

Diagnosis: Apothecia rounded to flexuose, often conglomerate, plane to moderately concave, yellowish brown; margin paler than the disc; ascospores acicular to filiform, 26–104 × c.2 µm.

Typus: Indonesia, Kalimantan Selan, Hulu Tabalong, 1°35’05’S, 115°31’27’E, old logged (c. 1983) dipterocarp forest, 630 m alt., 3 April 2000, *P. Wolseley T13 LQ* (BM 001104062 – holotypus! [TLC: No lichen substances detected; GenBank: XXXXXXXX]).

(Fig. 3B)

Prothallus thin to thick, white when young, later becoming brownish black. Thallus effuse, crustose, formed by small (up to 0.1 mm wide), adnate, isodiametric granules which are discrete to adjoined peripherally and soon form a more or less continuous crust; granules pale green and glabrous, sometimes slightly pubescent along the margin; isidia and soredia lacking; upper cortex composed of 1–2 layers of thin-walled hyphae with rounded cells, 5–10 µm thick; algal layer filling the inner part of the granules; cortex and algal layer not containing crystals. — Apothecia common, up to 1.2 mm diam., rounded or with a flexuose margin, simple or conglomerate, plane to moderately concave, yellowish brown; margin sometimes slightly pubescent when young, paler than the disc, rarely blackening on the underside; excipulum colorless throughout; hypothecium pale brown; epithecium colorless; no crystals in the apothecia; ascospores acicular to filiform, simple or with up to 3 pseudosepta, (26)–41–60–79–(104) × c. 2 µm (n=50). — Pycnidia not seen.

Distribution and ecology. Japan, Malaysia, and Papua New Guinea. Crustose on tree bark in pristine lowland primary forest at 160–1500 m alt.

Notes. Sporacestra longispora is similar to *S. straminea* by having yellowish brown apothecia but differs in having plane to concave apothecia, rather than plane to convex. It differs from *S. pertexta* by having yellowish brown, not reddish to purple brown apothecia; furthermore, it differs from both in forming longer, partly filiform ascospores

Etymology. Longispora, named after its delimiting character of long spores.

Additional species examined: **Japan:** Yaeyama Islands, Iriomote-jima island along the trail in the mountains along the Urauchi river from 500 m n of the southernmost starting point 6 km NW of Ohara village to the small

camping site between two streams 2.5 km NNE of the starting point (7.5 km NNW of Ohara village), Taketomi-cho, Yaegama-gun, 24°19'N, 123°51'E, 160-220 m alt., 1995, *G. Thor 13197, 13216* (UPS-L-392055, 392056). **Malaysia:** Malaysian Borneo, Sabah, Maliau Basin, 4.6972°N, 116.906°E, pristine lowland dipterocarp forest, 2012, *P. Wolseley, H. Thüs & C. Vairappan, M.87* (BORH). **Papua New Guinea:** Morobe, ridge SE of Bouwao, SW of Law, 147°0'E, 7°0'S, primary forest, on tree bark, 1500 m alt., 1965, *A.C. Jeremy 1499* (BM 000577330). Bulolo, Mt. Selk, 18 km from Bulolo on the way to the Lake, upphill, W of the road, 146°40'E, 7°15'S, secondary forest on tree bark, 750-850 m alt., *1965, D.J. Hil 12290* (BM 000921683).

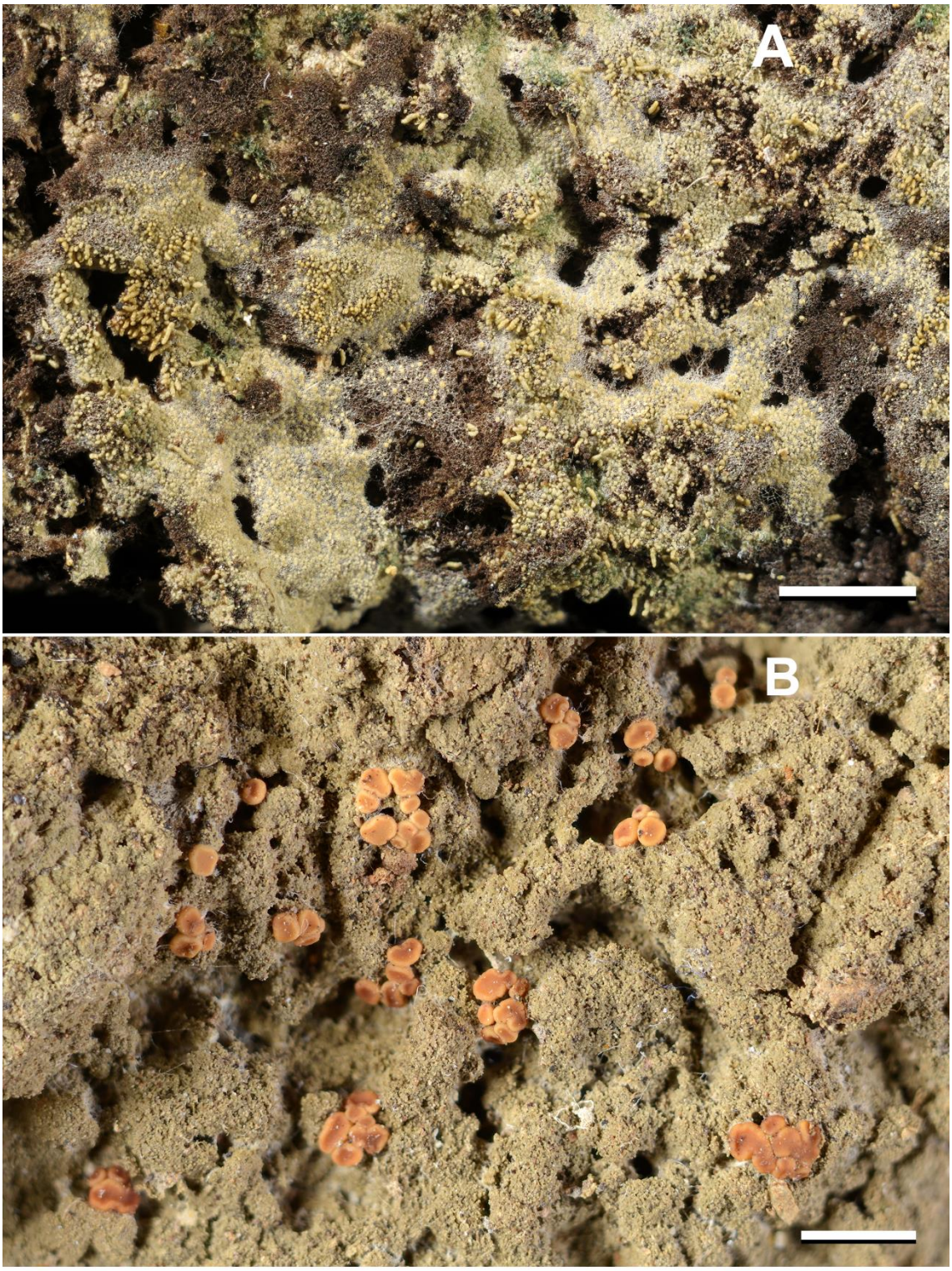

Fig. 3: (A) *Sporacestra isidiata* (holotype). Notice dark prothallus and cylindrical isidia; (B) *Sporacestra longispora* (holotype). Thallus with granular thallus and plane to concave apothecia. Scale bars = 2 mm. Photos by Einar Timdal.

Sporacestra pertexta (Nyl.) Stapnes & Timdal, comb. nov. [MB XXXXX].

≡ *Lecidea pertexta* Nyl. in Ann. Sci. Nat., Bot., ser. 4, 19: 347 (1863) ≡ *Psorella pertexta* (Nyl.) Müll. Arg. in Bot. Jahrb. Syst. 23: 297 (1897) ≡ *Phyllopsora pertexta* (Nyl.) Swinscow & Krog in Lichenologist 13: 244 (1989). Type: Cuba,"in ins. Cuba", *C. Wright. s.n*. (H-NYL 17344, holotype [TLC: No lichen substances, according to Timdal 2011]).

= *Biatora prasina* Tuck. & Mont. in Ann. Sci. Nat., Bot., ser. 4, 8: 296 (1857), nom. illeg. [non (Fr.) Fr. in Stirpes Agri Femsionensis: 38 (1826)] ≡ *Biatora prasinata* Tuck. in Syn. N. Amer. Lich. 2: 41 (1888). Type: Venezuela, s. loc. *A. Fendler s.n*. (H-NYL 21859a, isotype [image!]) ≡ *Bacidia prasinata* (Tuck.) Coppins in Bull. Br. Mus. Nat. Hist., Botany 11: 17-214 (1983).

= *Phyllopsora borbonica* Timdal & Krog in Mycotaxon 77: 68 (2001). Type: La Réunion, along road towards Plain d'Affoches, above Bras Citron, at point where road meets track. 20°57'S, 55°25'E, alt 1220 m, on tree trunk in forest. 1996-09-26 *H. Krog & E. Timdal RE8/12* (O-L-797, holotype! [TLC: No lichen substances, according to Timdal & Krog 2001; GenBank: XXXXXXXX]).

(Fig. 4A)

Prothallus medium thick, brownish black, light brown to white when young. Thallus effuse, crustose, formed by small (up to 0.1 mm wide), adnate, isodiametric granules which are discrete to adjoined peripherally and form a more or less continuous crust centrally; granules pale green and glabrous, often pubescent along the margin (at least when discrete); isidia and soredia lacking; upper cortex composed of 1–2 layers of thin-walled hyphae with rounded cells, 5–15 µm thick; algal layer filling the inner part of the granules; cortex and algal layer not containing crystals. — Apothecia abundant, up to 2 mm diam., rounded or with a flexuose margin, simple, plane to moderately convex (plane when young), reddish to purple brown; margin sometimes slightly pubescent when young, paler than the disc, blackening on the underside; excipulum pale brown to colorless in inner part, pale brown to dark reddish brown in the rim; hypothecium dark brown to reddish brown in the upper part, paler in the lower part; epithecium colorless; no crystals in apothecia; reddish brown pigment in excipulum and

hypothecium K+ purple; ascospores acicular, simple, with up to 3 pseudosepta, (17)–25–32–39–(53) × c. 2 µm (n=39). — Pycnidia not seen.

Distribution and ecology. I have verified specimens from: Brazil, Cuba, Mauritius, Papua New Guinea, La Réunion, and Venezuela. It is also reported from Samoa (Müller, 1897). The species is found on the bark in humid forests and humid woodlands at 530–1255 m alt.

Notes. Sporacestra longispora is morphologically similar, but differs in having lighter brown, more concave apothecia with a margin lighter colored than the disc. *Sporacestra straminea* differs from *S. pertexta* in having dull yellow apothecia.

Additional specimens examined: **Brazil:** Rio Abajo, 2014, *M. Cáceres, M. 20131, 20134* (ISE). **Cuba:** Pinar del Rio, La Palma, Magoto El Pan de Guajaibón, 22°47'N, 83°22'W, 705 m alt., *2006 S. Pérez-Ortega* (hb. Pérez-Ortega). **Mauritius:** Macchabee Forest (0.5–1 km ESE of Macchabee Kiosk), 20°24'S, 57°26'E, 600 m alt., 2006, *H. Krog & E. Timdal,MAU13/08* (O-L-21341). Rivière Noire District, Black River Gorges National Park; along trail to Piton de la Petite Rivière Noire, 20.42133°N, 57.41947°E, 630-700 m alt., 2016, *Diederich, P.,* (hb. Diederich 18432, 18454). **Papua New Guinea:** Morobe: Herzog Mts, Wago, ridge above village, 7°10'S, 146°40'E, primary forest, 1100 m alt., 1965, *A.C. Jeremy 4902* (BM 000577345). **Seychelles**: Mahé: Mare aux Cochons, on a Cinnamomum species, 1994, *K. Beaver,* (hb. Seaward 114397). **La Réunion**: Fôret de Bébour, along road towards Takamaka, by small bridge WSW of the end of the road, 21°05'S, 55°35'E, 1255 m alt., 1996, *H. Krog & E. Timdal RE30/11*(O-L-74322). Le Grand Etang, along the trail from the road to the lake, 21°05'S, 55°39'E, 530-540 m alt. 1996, *H. Krog & E. Timdal RE30/11* (O-L-74323).

Sporacestra straminea Stapnes, sp. nov. [MB XXXXX]

Diagnosis: Apothecia common, up to 1.5 mm diam., mostly rounded, simple, dull yellow; margin paler than the disc, sometimes blackening on the underside.

Typus : Brazil, Pará, Melgaço, Floresta Nacional de Caxiuanã, Estação Científica Ferreira Penna, 1°42.850'S, 51°27.207'W (WGS84), on tree trunk in tropical rainforest, 30–50 m alt., 2015-03-14, *S. Kistenich & E. Timdal SK1-097* (O-L-201106, holotype! [TLC: No lichen substances detected; GenBank: XXXXXXXX]).

(Fig. 4B)

Prothallus thin to thick, white when young, later becoming brownish black. Thallus effuse, crustose, formed by small (up to 0.1 mm wide), adnate, isodiametric granules which are discrete to adjoined peripherally and soon form a more or less continuous crust; granules pale green and glabrous, sometimes slightly pubescent along the margin; isidia and soredia lacking; upper cortex composed of 1-2 layers of thin walled hyphae with rounded cells, 5-10 µm thick; algal layer filling the inner part of the granules; cortex and algal layer not containing crystals. — Apothecia common, up to 1.5 mm diam., mostly rounded, simple, plane to moderately convex, dull yellow; margin sometimes slightly pubescent when young, paler than the disc, sometimes slightly blackening on the underside; excipulum colorless throughout; hypothecium pale brown; epithecium colorless; no crystals in apothecia; ascospores acicular, simple or with up to 3 pseudosepta, (38)–47–53–58–(65) × c. 2 µm (n=40). — Pycnidia not seen.

Distribution and ecology. Brazil. On tree trunks in Amazonian rainforests.

Notes. The species resembles both *S. longispora* and *S. pertexta* in most of its external and internal morphological characters, but differs from both in the appearance of the apothecia. The color of the apothecia, dull yellow, can resemble apothecia in *S. longispora*, but the disc is convex rather than concave. The species differs from *S. pertexta* in the color of the apothecia, whereas *S. straminea* has dull yellow apothecia, *S. pertexta* has reddish to purple brown.

Etymology. Straminea, named after its dull yellow apothecia.

Additional specimens examined: **Brazil:** Pará, Melgaço, Floresta Nacional de Caxiuanã, Estação Científica Ferreira Penna, 1°42.847’S to 1°44.332’S, 51°27.200’W to 51°27.667’W, on tree trunk in tropical rainforest, 30-50 m alt., 2015-03 *Kistenich, S. & Timdal, E., SK1-011, -024, -039,-092* (O-L-201020,-201033,-201048,-201101). Pará, Tailândia, Fazenda Agroecológica São Roque, 3°04.501’S, 49°01.0881W, on tree trunk in tropical rainforest, 50 m alt., 2015-11 *Dahl et al. SK1-316* (O-L-202596).

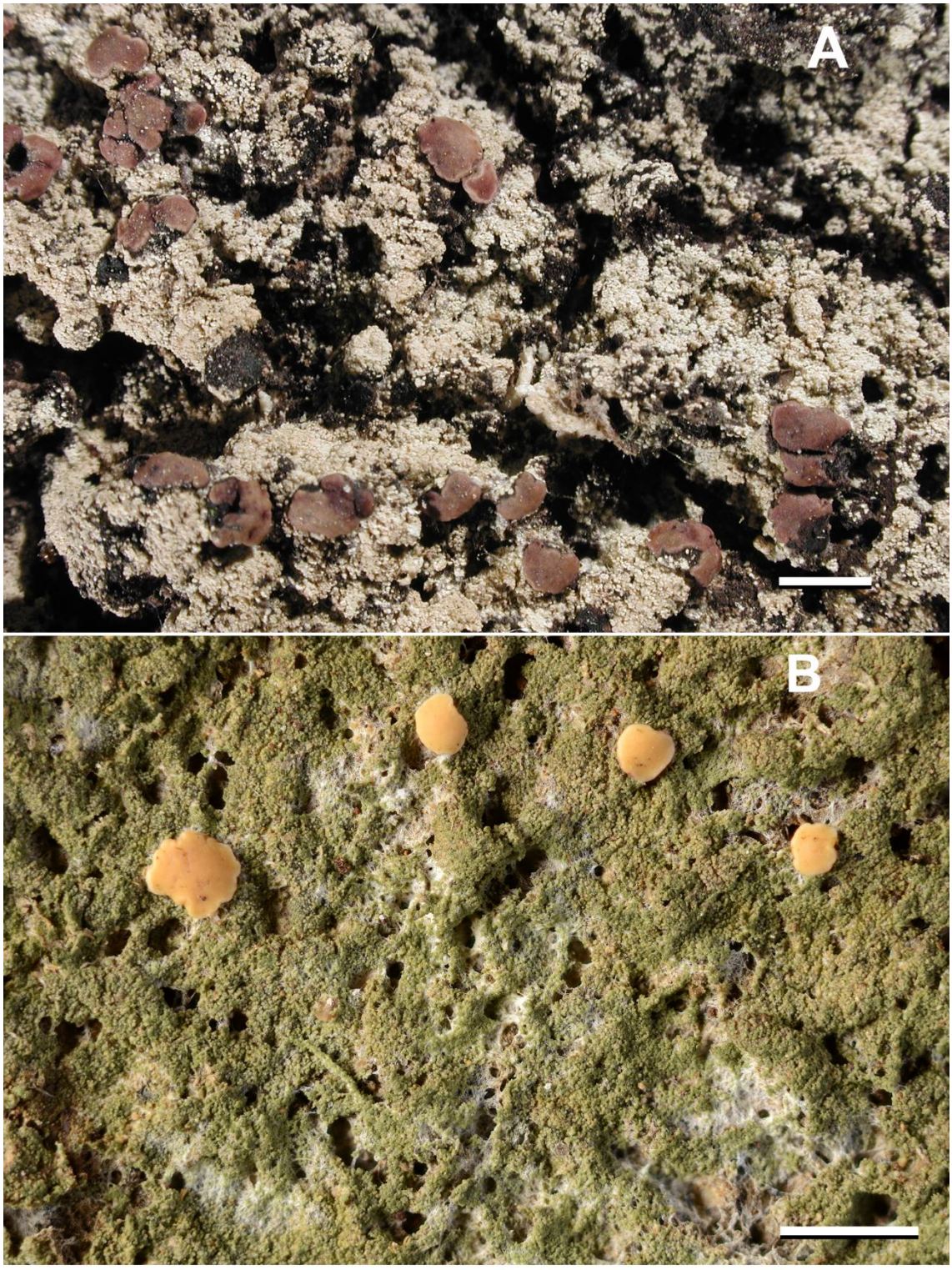

Fig. 4: (A) *Sporacestra pertexta* (holotype of *P. borbonica*). Notice the granular thallus with brown apothecia; (B) *Sporacestra straminea* (holotype). Notice the young white prothallus, and green thallus with dull yellow apothecia. Scale bars = 2 mm. Photos by Einar Timdal.

Appendix: *Bacidia* De Not.

In Giorn. Bot. Ital. 2: 189 (1846). Type: *Bacidia rosella* (Pers.) De Not.

Bacidia corallina Stapnes & Timdal, sp. nov. [MB XXXXX]

Diagnosis: Similar to the *Bacidiopsora* group of *Bacidia* but with coralloid isidia.

Typus: Thailand, Uthau Thani. Site: Kapu Kapiang station, Khlong Plou, 99°16'E, 15°39'N, in dry evergreen forest, 500 m alt., 1993-02, *Aguirre, James & Wolseley 2478a* (BM 000749861, holotype! [TLC: Hyperhomosekikaic acid and/or homossekikaic acid and unidentified xanthone detected; GenBank: XXXXXXX]).

(Fig. 5A)

Prothallus thick, black. Thallus corticated, formed by small, adnate, isodiametric granules up to 0.3 mm diam., remaining discrete; granules pale greenish gray (herbaria material); isidia abundant, attached marginally to the granules, coralloid, c 0.03 mm wide, up to 0.4 mm long; upper cortex up to 10 µm thick, composed of 2–3 layers of thick-walled hyphae with rounded lumina, not containing crystals; algal layer filling the inner part of the granules, densely incrusted by crystals refracting polarized light and dissolving in K; — Apothecia common, up to 1.2 mm diam., rounded, plane to slightly convex, yellowish pale brown, blackening on the underside, not pubescent; proper excipulum composed of radiating, conglutinated, thick-walled hyphae with broadly cylindrical to ellipsoid lumina, reddish brown in the rim (K+ purpur), pale brown in the inner part, containing crystals refracting polarized light; hypothecium yellowish brown, not distinctly delimited from exipulum, chondroid, composed of irregularly oriented, thick-walled hyphae with cylindrical lumina, not containing crystals; hymenium colorless (with amyloid gelatin), 100–120 µm high; paraphyses conglutinated, straight, simple or rarely branched, c. 4 µm wide, with a slightly swollen, colorless apical cell; ascus narrowly clavate, with a well-developed tholus; tholus amyloid in upper part, lacking ocular chamber and axial mass; ascospores acicular, straight or slightly curved, parallel in the ascus, ± 7-septate, colorless, (39)–49–55–62–(65) × c. 2 µm (n=15). — Pycnidia unknown.

Chemistry. Hyperhomosekikaic and/or homosekikaic acid and xanthones.

Notes. The species is similar to members of the *Bacidiopsora* group of *Bacidia*, but differs in containing additional xanthones, having a granulose thallus with coralloid isidia, yellowish brown apothecia, and crystals only in the excipulum, not in the hypothecium. The excipulum is thinner than in *Bacidiopsora* and is composed of hyphae with shorter, more broadly cylindrical to ellipsoid lumina.

Distribution and ecology. Thailand. Crustose on bark in dry evergreen forest at 500 m alt.

Etymology. Corallina, named after the coralloid isidia.

Additional specimens examined: **Thailand:** Uthai Thani. Site: Kapou Kapiang station, Khlong Plou, 99°16'E, 15°39'N, in dry evergreen forest, 500 m alt., 1993-02, *Aguirre, James & Wolseley 4277e* (BM 001031544). Khao Nang Rum Viewpoint track 2.2, 99°18'E, 15°29'N, in dry everegreen forest, 370-460 m alt., *Aguirre, James & Wolseley 2479a, 2715* (BM 000749844, 000749853).

Bacidia kariegae Stapnes, sp. nov. [MB XXXXX]

Diagnosis: Similar to the *Bacidiopsora* group of *Bacidia* but with granular soralia.

Typus: South Africa, Eastern Cape, Western District: 11 km NNW of Kenton-on-Sea, 33°35.36'S, 26°37.31'E, on tree trunk in low, dry forest, 160 m alt., 2015-09, *S. Rui & E. Timdal13875* (O-L-202868, holotype! [TLC: Hyperhomosekikaic and/or homosekikaic acid detected; GenBank: XXXXXXXX])

(Fig. 5B)

Prothallus, thick, black. Thallus corticated, formed by small, ascending, irregularly imbricate, isodiametric squamules up to 0.5 mm diam.; squamules dull, pale green, soon breaking in to effuse, granular soralia; upper cortex up to 15 µm thick, composed of 2–3 layers of thick-walled hyphae with rounded lumina, not containing crystals; algal layer up to 20 µm thick; medulla densely incrusted by crystals refracting polarized light and dissolving in K. — Apothecia and pycnidia not seen.

Chemistry. Hyperhomosekikaic acid and/or homosekikaic acid.

Notes. The species resembles *Bacidia microphylla* and differs mainly in forming granular soralia.

Distribution and ecology. South Africa, known from the type locality only. On tree trunk in low dry forest.

Etymology. Kariegae, from the type locality in the Kariega Game Reserve.

Additional specimens examined: **South Africa**, Eastern Cape, Western District, 11 km NNW of Kenton-on-Sea, 33°35.35'S, 26°37.23'E, on tree trunk in low, dry forest, 160 m alt., 2015-09, *S. Rui & E. Timdal13874* (O-L-202867).

Bacidia microphylla Stapnes, nom. nov. [MB XXXXX]

≡ *Bacidiopsora microphyllina* Kalb in Biblioth. Lichenol. 88: 304 (2004) [non *Bacidia microphyllina* (Tuck.) Riddle, Mycologia 15: 80 (1923)].

Note. The species is here reported new to South America.

Additional specimens examined: **South Africa:** Mpumalanga, Ehlanzeni District, Buffelkloof Nature Reserve, 25°16.12'S, 30°30.97'E, on tree in mist forest, 1700 m alt., 2014-10, *J. Burrows & E. Timdal, 14204, 14214, 14219* (O-L196747, 196757, 196762). **Venezuela:** Capital District, Parque Nacional Waraira Repano, 10°32.827'N, 66°52.629'W, on tree trunk in tropical moist Forest, 1800 m alt., 2015-11, *Dahl et al., SK1-204, SK1-205* (O-L-202484, 202485).

Bacidia soralifera Stapnes & Timdal, nom. nov. [MB XXXXX]

≡ *Triclinum sorediatum* Aptroot & Sparrius in Aptroot et al., Fungal Diversity 24**:** 130 (2007)
≡ *Phyllopsora sorediata* (Aptroot & Sparrius) Timdal in Lichenologist 40: 337 (2008) [non *Bacidia sorediata* Lendemer & R.C. Harris, Bryologist 119: 167 (2016)].

(Fig. 5C)

Prothallus white to pale grey, poorly developed. Thallus effuse, crustose to subsquamulose, formed by medium sized (up to 0.3 mm wide) plane to weakly convex, adnate or partly ascending, isodiametric areolae or squamules; areolae and squamules scattered when young, later becoming contiguose or ascending and irregularily imbricate, greyish green (according to Aptroot et al. 2007), not pubescent; soralia punctiform when young, later irregular and

often confluent, granular, white; upper cortex up to 15 µm, composed of more or less irregularly arranged, thick-walled hyphae with angular lumina, containing crystals refracting polarized light; algal layer filling the inner part, containing smaller crystals; photobiont green unicellular algae, up 10 µm diam. — Apothecia common, up to 1 mm diam., mostly rounded, simple, weakly concave to moderately concave, dull yellow to brownish yellow; margin thick when young, later becoming more indistinct, paler than the disc, not blackening on the underside; excipulum colorless with crystals; hypothecium colorless; epithecium colorless with a few scattered crystals; ascospores acicular, straight or slightly curved, simple or with irregular pseudosepta, (18)–22–27–33–(44) × c. 2 µm (n=40). — Pycnidia not seen.

Chemistry. Atranorin, divaricatic acid and two unknowns.

Notes. A full description based on my investigation of material collected at the same locality and date as the holotype is given here because the original description of Aptroot et al. (2007) seems to be based on mixed material (i.e., the specimen containing fumarprotocetraric acid). The species is here reported as new to Africa.

Distribution and ecology. Known from South Africa and Thailand, crustose on bark in dry dipterocarp forest at 460–640 m alt.

Additional specimens examined: **South Africa:** Eastern cape, Grootrivier (1km N of Natures Valley not far from the bridge), 33°58'S. 23°34'E, on trunk of Podocarpus falcatus, 10 m., *Anders Nordin, 4642* (UPS L.92604) **Thailand:** Uthai Thani. Site: Kapou Kapiang station, khlong Plou, 99°11'E to 99°16'E, 15°30'N to 15°39'N, on bark in dry evergreen forest, 500 m alt., 1993-03, *Aguirre,, James & Wolseley, 2806, 2854, 2877, 2906* (BM 000749892, 000763640, 000749760, 000749761). Khao Nang Rum Viewpoint track 205, 99°18'E, 15°29'N, on bark in dry dipterocarp forest, 460-640 m alt., 1992-01, *B. Aguriee-Hudson & P.A. Wolseley, 1397, 3948* (BM 000749859, 000749765).

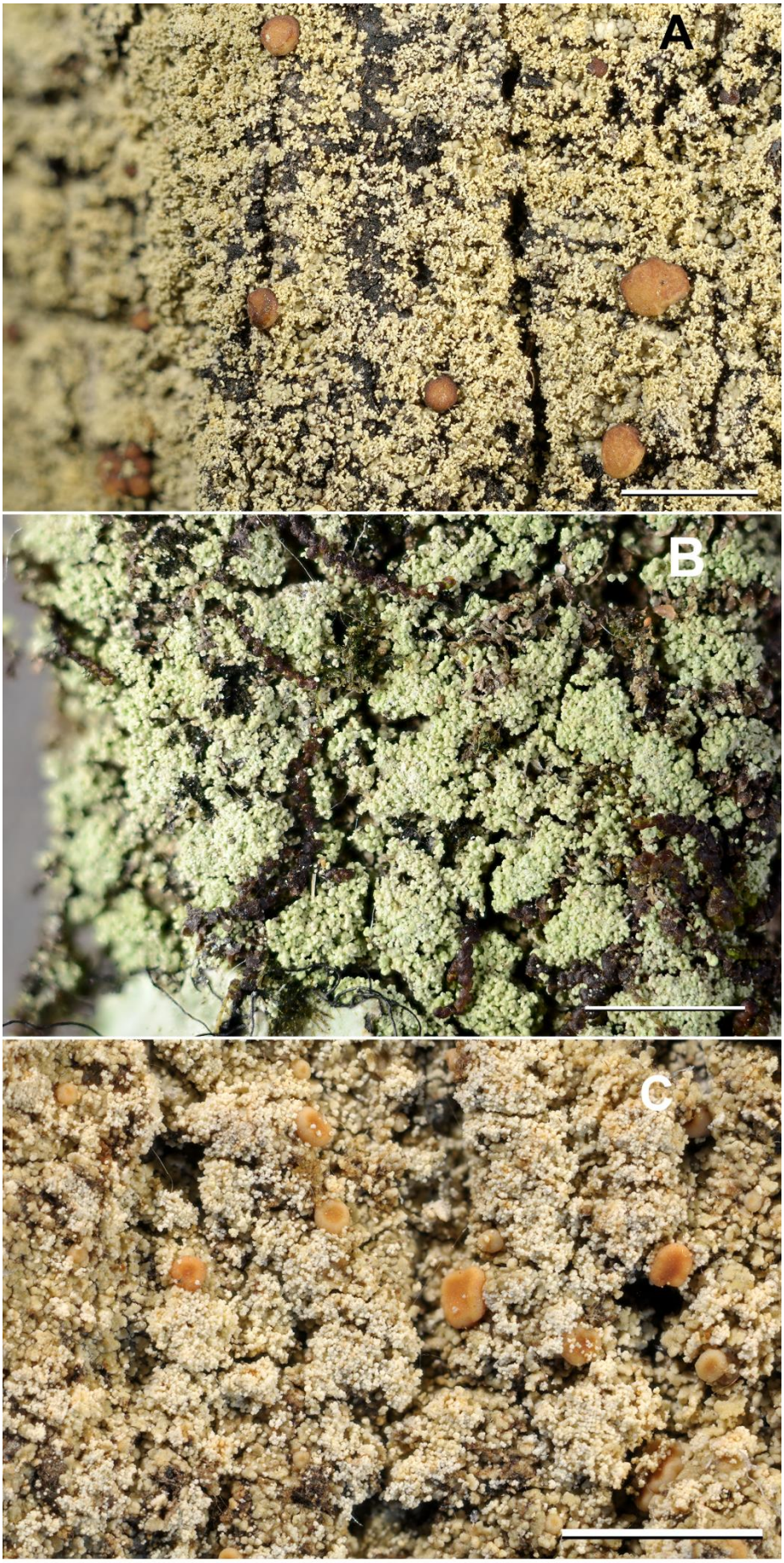

Fig. 5: **(A)** *Bacidia corallina* (holotype). Notice the thallus with apothecia and coralloid isida; **(B)** *Bacidia kariegae* (holotype). Notice the squamulose thallus dissolving into granular soredia; **(C)** *Bacidia soralifera* (BM 000749893). Notice the areolated thallus with apothecia and soredia. Scale bars = 2 mm. Photos by Einar Timdal.

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Appendix

Figure S1: PJ phylogeny of sequence alignment 2 (A) and 3 (B).

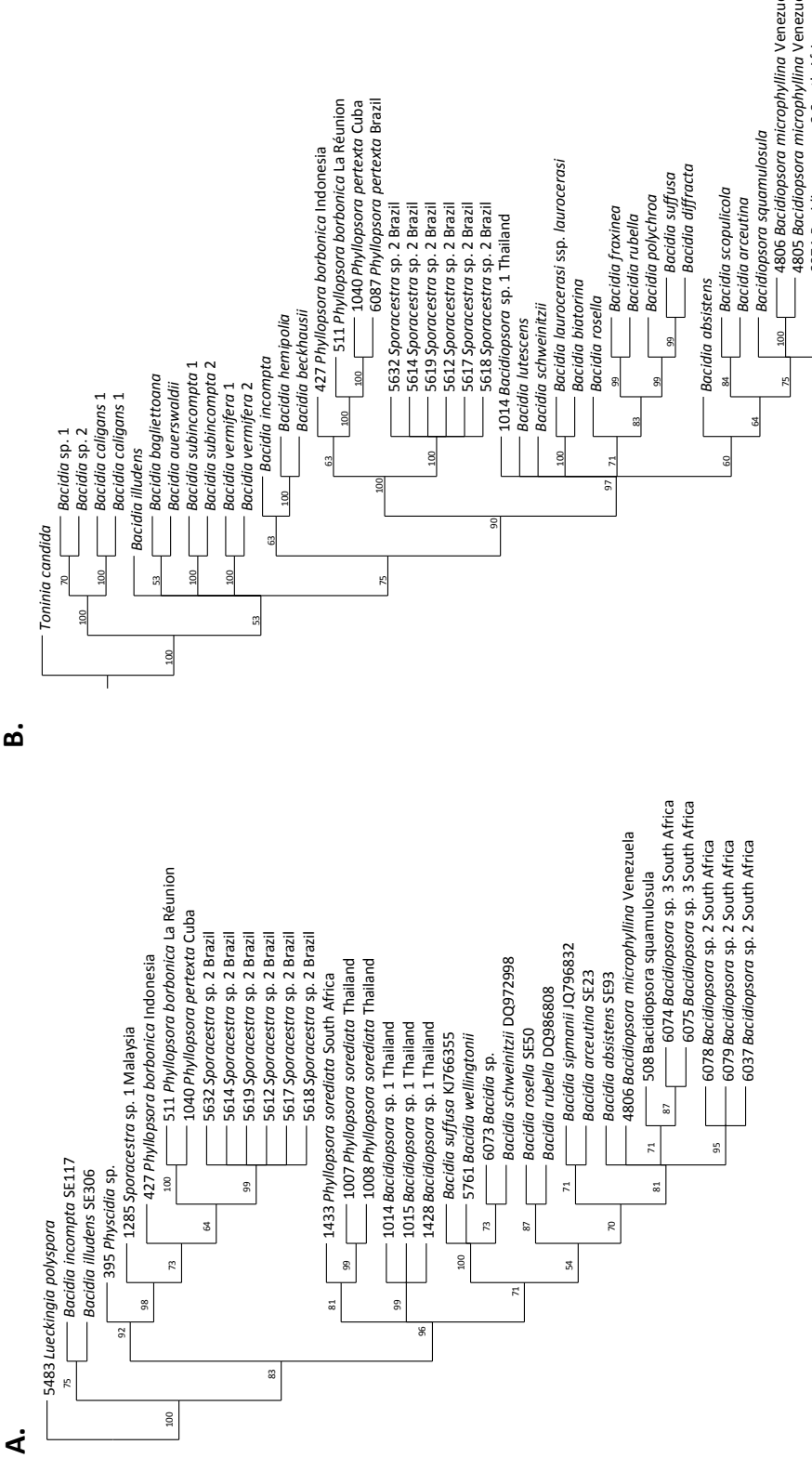

Fig. S1: two parsimony jackknife (PJ) consensus phylogenies. **(A)** Is based on 34 accessions and a 998 basepairs long alignment (sequence alignment 2) from one mitochondrial (SSU) marker. **(B)** Is based on 42 accesions and a 610 basepair long alignment (sequence alignment 3) from one nuclear (ITS) marker. Both phylogenies consists of accessions from *Bacidia*, *Bacidiopsora*, *Phyllopsora* and *Sporacestra*. Jackknife (JK) branch support are reported above branches.

Figure S2: PJ phylogeny of sequence alignment 4

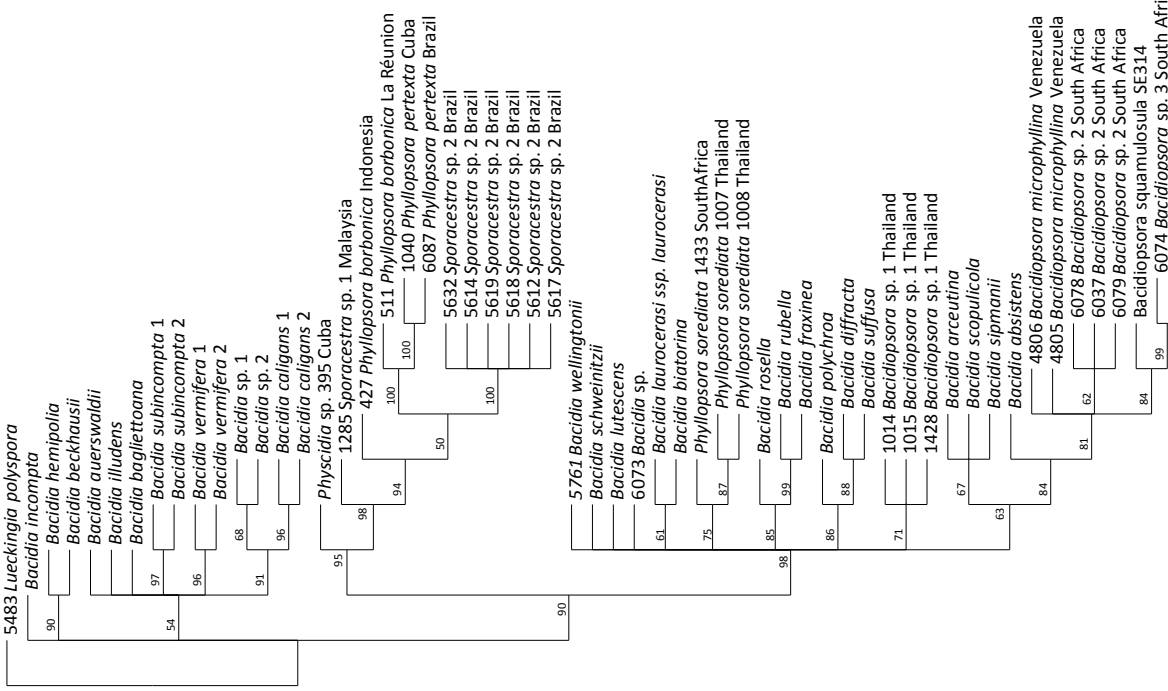

Fig. S2: The parsimony jackknife (PJ) consensus phylogeny based on 57 accessions and a 1608 basepairs long alignment from one nuclear (ITS) and one mitochondrial (SSU) marker. It consists of accessions from *Bacidia*, *Bacidiopsora*, *Phyllopsora* and *Sporacestra*. Jackknife (JK) branch support are reported above branches.